BOTRYTIS SPORES TRANSFER CHARACTERISATION AND MODELLING IN A ROSE GREENHOUSE

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Abstract

Fungal pathogens and especially grey mould are among the most virulent bioagressors of protected cultivations. Inside greenhouse climate marked by high air temperature and humidity content is highly favourable to the development of *B cinerea* and its control is further complicated by the absence of commercial resistant varieties and a limited arsenal available for chemical control. This fungus is very prolific and has a rapid asexual reproductive cycle marked by an abundant spore production which is easily transported by air movements and may contribute to the development of explosive epidemics.

The present study will focus on the determination of the balance of the spores inside a rose greenhouse in order to determine if the spores are in majority produced inside or outside the greenhouse and to predict their displacements, deposition and interception by the crop cover.

Two major approaches have been undertaken:

- An experimental one, based on the Botrytis spore balance of the finite greenhouse volume, spore concentration beings considered as a particular species which is transported by air in the same way as heat, CO2, water vapour or any tracer gas, spore balance being performed in the same way as for these species.

- A modelling approach, based on the development of specific module associated to a conventional Computational Fluid Dynamics model, to model, compute and solved the transport and deposition of *B. cinerea* spores on vegetation rows, soil and insect-proof nets.

First experimental results are presented for young rose crop cultivation. It is shown that the origin of the inoculums is predominantly external but that internal spore's production increases as the plantation grows old. These experimental data are used for the validation of the CFD based spore transfer model and, once validated the CFD model of spores transfer is used to examine the distribution of the concentration and the deposition of spores inside the greenhouse. Various improvements aiming at spore control are deduced and discussed.

Introduction

Grey mould caused by *Botrytis cinerea* and more generally all the airborne fungal pathogens are considered as a major problem in greenhouse production of ornamentals and vegetables. As microclimatic parameters have long been recognised as key factors in the development of diseases caused by fungal pathogens, greenhouse climate management in relation to disease control is another alternative to control pests efficiently (Nicot and Baille, 1996; Tantau and Lange, 2003). However, it requires also determining the fungal inoculums. Recent progress in the fields of greenhouse climatology allows for the calculations (Boulard *et al.*, 2002) and the control (Boulard *et al*., 2004) of the climate and particularly air humidity at the phylloplane, in the leaf boundary layer. However the other component of the question, the fungal inoculum in greenhouses, remains poorly known and documented. An efficient crop protection against these airborne organisms requests to determine their origin, i.e. if they come from outside or if they are produced inside the greenhouse and of course their rate of production.

We shall describe two approaches aiming at the determination of the spore's transfer, i) the adaptation of the classical methods used for performing gas or heat balances in finite volumes such as greenhouses, to try performing a *Botrytis cinerea* spore's balance and ii) the

use of a appropriately modified Computer Fluid Dynamics (CFD) models for performing distributed models of spore's transfers in the greenhouse and between the greenhouse and its environment. We present in this paper a study aiming at this determination, for a rose plastic house located in the South of France near Nice.

Theory

Whole greenhouse spore's balance

In this study we have considered the finite volume of the whole greenhouse and we have performed its spores balance (as for a gas balance) to finally assess the inside production of inoculums or its entry from outside. After an experimental determination of the greenhouse air exchange rate G (m^3s^{-1}), we have considered and experimentally determined the various elements of the spores balance (fig. 1).

If C_i (t) and C_e(t) are respectively inside and outside *Botrytis cinerea* spores concentration (sp) at time t (s),V the whole greenhouse volume (m^3), D_i (sp s^{-1}) the spore deposit within the greenhouse by sedimentation or impaction and P_i (sp s^{-1}) the inside spore production, the greenhouse spore content at time (t+1) will be equal to the initial spore content at time t (first right side member of relation *(1)*), minus the exit of inside spores during the considered time interval Δt (second right side member of relation (1)) plus the entries of outside spores during the considered time interval Δt (third right side member of relation *(1))*, minus the spores deposition on the soil and on the greenhouse crop during the considered time interval Δt (fourth right side member of relation *(1))*, plus, finally, the inside spore production during the considered time interval Δt (last right side member of relation (1)).

$$VC_i(t+1) = VC_i(t) - GC_i\,\Delta t + GC_e\,\Delta t - D_i\,\Delta t + P_i\,\Delta t \qquad (1)$$

Measuring the inside and outside spore concentration (C_i *(t)* & C_e*(t)*) together with the spore deposit D_i (we don't consider the spore's moving again to the air because of air movement because spores are stick in the growing media in the Petri dishes) and determining the air exchange rate G by means of available global ventilation models and knowing the greenhouse volume (*V*), one's can deduce the rate of inside spore production P_i for any time scale Δt ranging approximately between 1 and 24 hours. A complete presentation of the whole greenhouse spore's balance can be found in Boulard *et al.* (2006).

CFD model of the spore's transfer

Computational Fluid Dynamics constitutes nowadays a powerful tool for the determination of the climatic parameters prevailing in a greenhouse system. From an agronomic point of view (Quinn *et al.*, 2001), the determination of the prediction of the concentration pattern of pollutants or biotic materials is of big interest, especially for the determination of the concentration of spores transported by wind and deposited or removed on leaf surface in greenhouse crop. For that purpose, the simulation of the concentration of spores can be achieved with an Eulerian model that describes the transport of a scalar (the concentration of spores) in the domain of interest (the greenhouse and its direct environment). The boundary conditions must be accurately defined for the deposition rate of spores on the greenhouse soil and for the interception rate of spores by the insect-proof screens located in the greenhouse vents. The influence of the crop, considered as a porous medium for the momentum equation, is taken into account by using the impaction rate of spores on the plant.

We presents the first results of 2D numerical simulations performed with the Fluent CFD code for a multi span greenhouse with roof vents and a side opening both equipped with insect-proof nets and we focus more particularly in this to the validation of the modeling of deposition of spores on soil and of interception of spores within the crop.

The CFD code Fluent v. 6.1. has been used to perform the simulations of the flow in the greenhouse. The determination of temperature and humidity fields within the greenhouse is necessary to correctly compute the velocity fields. The evapo-transpiration model for the

crop is taken into account in the solver as source terms for the equations of energy and water vapour concentration (Boulard & Wang, ; Roy & Boulard, 2004) and the standard k-ε model has been selected to model the turbulence effects in the airflow. Therefore, the solved variables are: momentum components, pressure, turbulent energy, dissipation rate of turbulent energy, temperature, humidity and concentration of spores in the domain. A specific routine (User Defined File) has been specially developed to take into account the source terms of spores in specific zones. Details on the domain together on the transport and deposition of spores equation and numerical solving can be found in Roy et *al.* (2006).

Experimental set up

The greenhouse: the experimentations have been carried on in a three spans compartmented plastic house (Multiclair 9600, Filclair, France). The main compartment (576 m^2) was occupied by a soiless rose plantation (3240 rose plants, cv Magnum, Milva, Suella). Ventilation was performed by means of roof openings and a side opening, both equipped with insect proof nets (Anti-Bemisia 10x20 meshes/cm) with a 0.2 mm thread diameter and a 59% solidity.

Greenhouse ventilation performances were achieved using tracer gas measurements by means of the decay rate technique with N_2O as tracer gas. Measurements were performed for the two prevailing wind directions and the corresponding values of the wind dependent coefficient of ventilation used in the ventilation model (Boulard & Baille, 1995) were deduced. Details on the air exchange rate procedures and measurements for this greenhouse can be found in Fatnassi *et al.* (2006).

Climate measurements: Wind speed and direction together with inside and outside temperature and humidity and vents opening area were systematically measured by means of the sensors of the greenhouse climate computer (L'ien, Vitrolles, 13, France) with a 1 minute frequency before being stored in the climate computer and processed.

Spores balance measurements:

Inside (C_i) and outside (C_e) *Botrytis cinerea* spores concentration was measured by means of 2 spores trap systems (Burkard Manufacturing Co Ltd, Rickmansworth, GB) with a $0.6 m^3.h^{-1}$ flow rate, disposed at about 1.5 m from the soil surface over the crop cover (fig. 2). These spore traps were continually operated during the trials and the spores captured in the air were disposed every day on a selective medium (Kerssies, 1990) and counted using a binocular after a 10 days incubation period.

The total spores deposit in the greenhouse (D_i) was estimated by means of 16 Petri dishes horizontally disposed (fig. 3) to measure the spore sedimentation over the plant cover together with 20 Petri dishes oriented in the 4 directions to sense the vertical impaction (fig.3). As for the spores traps, these dishes were disposed every day on a Kerssies medium and counted using after a 10 days incubation period

Results

Spores balances on a daily basis (24h) have been performed during 4 trials in October 2003, April and November 2004 and May 2005, each trial lasting between 3 and 4 weeks. Table 1 summarises the different terms of the balance for all the trials and more specifically for the April 2004 and May 2005 trials.

Spore balance calculations

Figure 4 give an example of the terms of the spore balance for the May 2005 trial. One can state than during the measurement period (14 days), the spore production is systematically positive with even spore's exportation from inside to outside the greenhouse as shown by the negative value, from time to time, of the exchanges with outside.

If we compare April 2004 and May 2005 trials, (Table 1), we can see that the spore concentration difference between inside and outside can vary a lot from one period to another: inside concentration is about three times less important than outside for the April 2004 trial (0.8 vs 2.7 spm^{-3}) whereas it is approximately the same for the May 2005 trial. Figure 5 shows that generally, inside spores concentration remains stable from one period to the other (0.8 spm^{-3}) whereas the outside concentration varies a lot (from 0.8 to 2.7 spm^{-3}). On average for the four trials (Table 1), two thirds of the deposited spores come from inside production and one third from outside one. However important evolutions can be observed from one period to the other, as illustrated by the comparisons between the different terms of the balance for the April 2004 and May 2005 trials (fig 6).

CFD model of the spore's transfer

The simulated velocity vector and spore's concentration fields for inside and outside the greenhouse resulting from the CFD modelling are presented in figs.7 and 8. For the outdoor part of the domain, the concentration of spores has been set up to 6 spores m^{-3} and is homogeneous, expect in the vicinity of the ground where the concentration is divided by 2. Hence the concentration flowing in the left span through the side vent is about 4.5 spores m^{-3} and is reduced to 2 spores m^{-3} directly after the insect proof screens which are deployed in the vent openings. From the left span to the third span, the concentration of spores is reduced from 2 to 0.33 spores m^{-3} under the effects of impact and sedimentation within the crop cover. The flux of captured spores per volume unit within the crop is presented in fig.9. It decreases from 2.9 10^{-2} to 5 10^{-3} spores m^{-3} s^{-1}, the diminution being linked to both concentration and velocity diminution from the left to the right of the crop.

For validation the mean concentration of spores within the three left spans has been computed from the simulation results (Fig. 10) and is equal to 1.13 spores m^{-2} and compared with the concentration which was measured within the crop (1.05 spores m^{-3}).

Discussion and conclusion

Two approaches aiming at the determination of the spore's transfer inside a greenhouse have been used, i) based on experimental measurements, a global *Botrytis cinerea* spore's balance of the finite volume of the greenhouses and ii) the use of a appropriately modified CFD model for performing distributed models of spore's transfers in the greenhouse and between the greenhouse and its environment.

The first attempt to perform a whole greenhouse *Botrytis cinerea* spore's balance inside a greenhouse (case i) gives likely results and allows determining the origin and the evolution of the inoculums.

Likewise the Fluent CFD code has been used to develop an Eulerian model for the simulation of the concentration of the spores together with the use of an active crop model for the determination of the velocity, temperature and humidity patterns in the domain of interest. Numerical results for the concentration of spores show also a good accord with the experimental measurements for the inner concentration and the inside distribution of spore.

It is now expected to exploit this model for studying both biotic and abiotic spore's production dependence and more generally of the *Botrytis cinerea* activity within the greenhouse.

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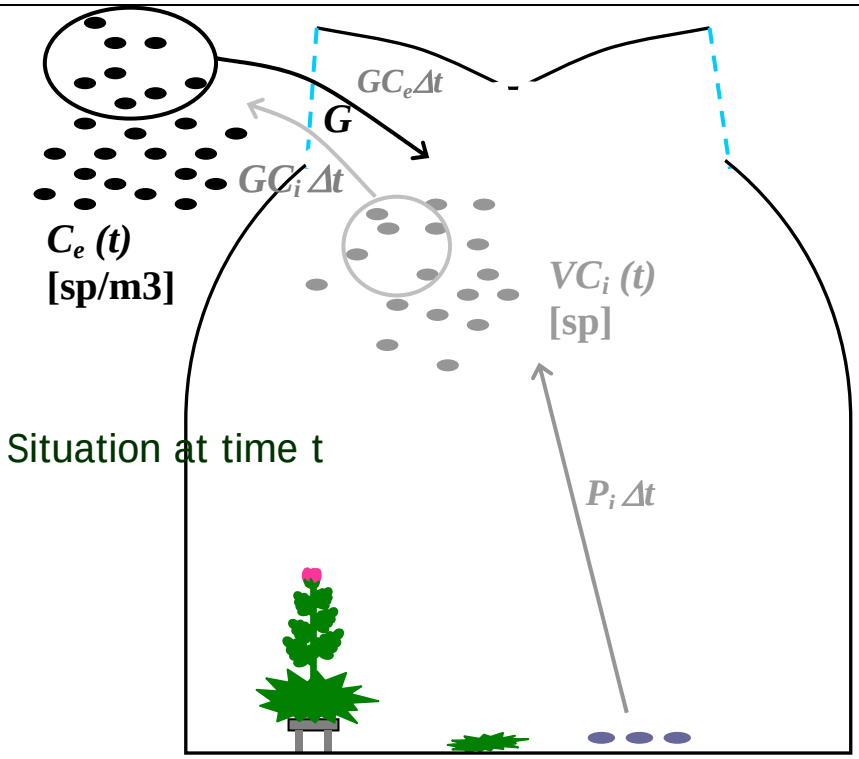
Variables	Average of the 5 trials	April 2004 trial	May 2005 trial
Variables	Mean value	Mean value	Mean value
G (m^3s^{-1})	2.4	1.7	7.9
C_e(spm^{-3})	1.8	2.7	0.8
C_i(spm^{-3})	0.8	0.8	0.9
D_i (sps^{-1})	1.4	1.9	2.9
FG(C_e- Ci) Δt	35853	104197	-14615
$VdC_i+D_i\Delta t$	122083	160231	249713
$P_i\Delta t$.	85939	54891	266329

Table 1: Main components of the *Botrytis cinerea* spores balance.

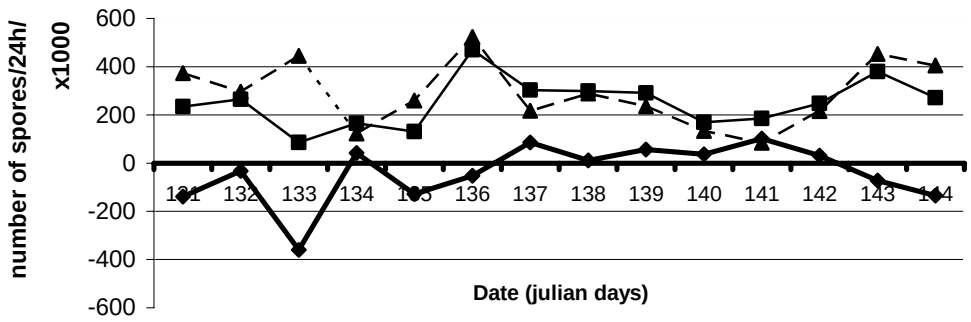


C_e : Outside air concentration in spores of *B. cinerea*
C_i : Inside air concentration in spores
P_i : Inside production of spores
D_i : Spores deposition on greenhouse soil or on crop cover
V : Greenhouse volume
G : Air exchange rate
Δt : Time space

Fig. 1 : Method for studying the origin of the *Botrytis cinerea* inoculum's using a spores balance

Fig. 2: Photo of the inside spore trap Burkard Manufacturing Co Ltd

Fig. 3: Photo of one horizontal Petri dishe, to measure spore sedimentation and of a vertical one, to measure spore impaction.



c)

Figure 4 : Variations of the main terms of the spores balance: the exchange with outside $FG(C_i-C_e)\ \Delta t$: ♦; the spores deposition $V(C_i(t+1)- C_i(t))+D_i\Delta t$: ■, and the spore production $P_i\Delta t$: ▲, on a 24h time scale basis: for the May 2005 trial.

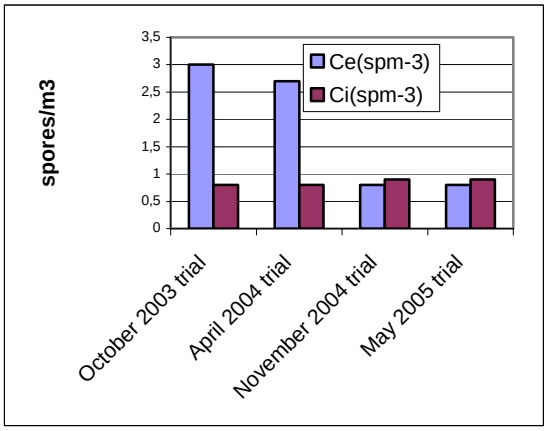


Fig. 5: Average values for measured inside and outside spores concentration for the different trials.

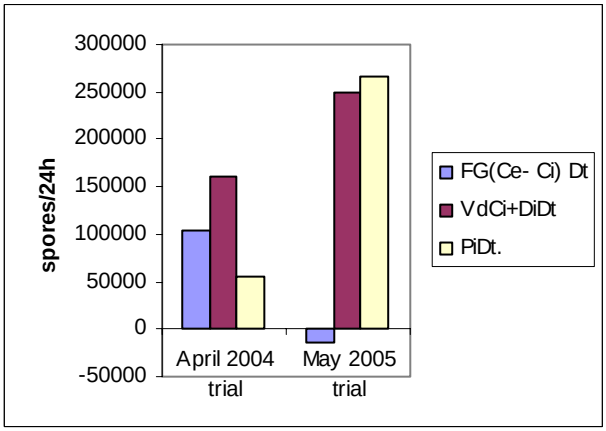


Fig. 6: Average values of the different terms of the *Botrytis cinerea* spores balance for the April 2004 & May 2004 trials.

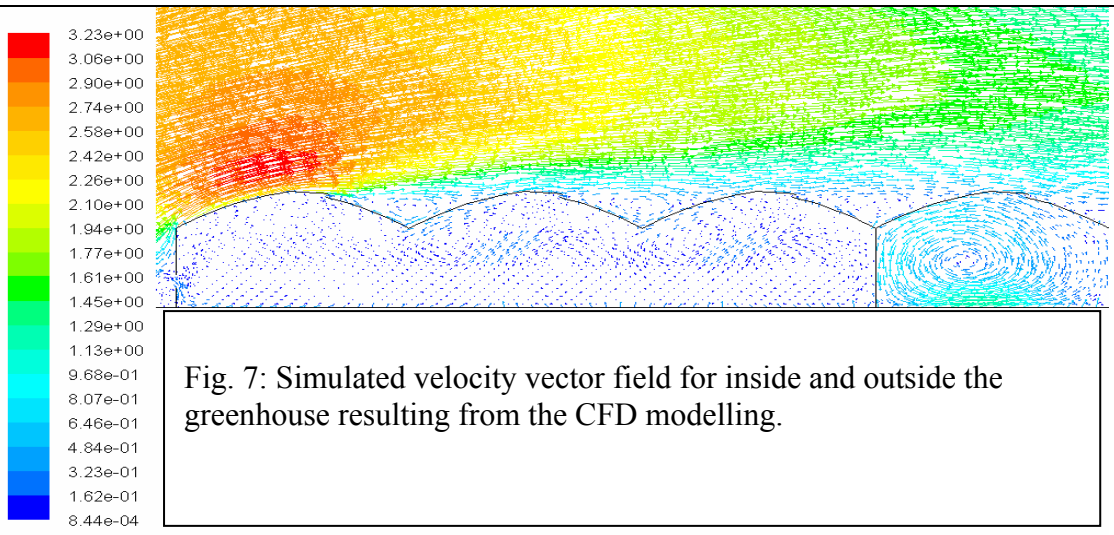


Fig. 7: Simulated velocity vector field for inside and outside the greenhouse resulting from the CFD modelling.

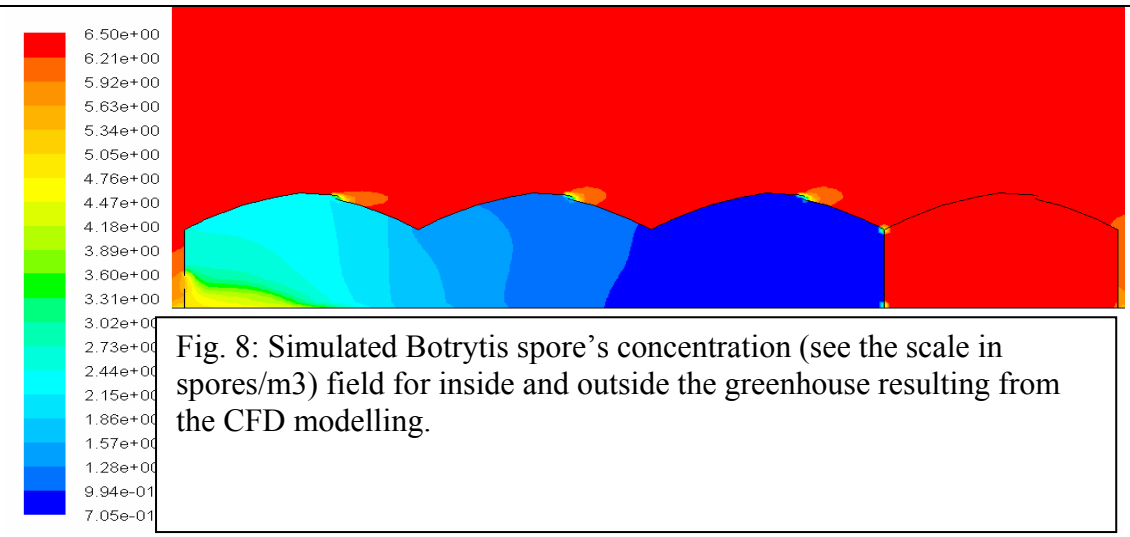


Fig. 8: Simulated Botrytis spore's concentration (see the scale in spores/m3) field for inside and outside the greenhouse resulting from the CFD modelling.

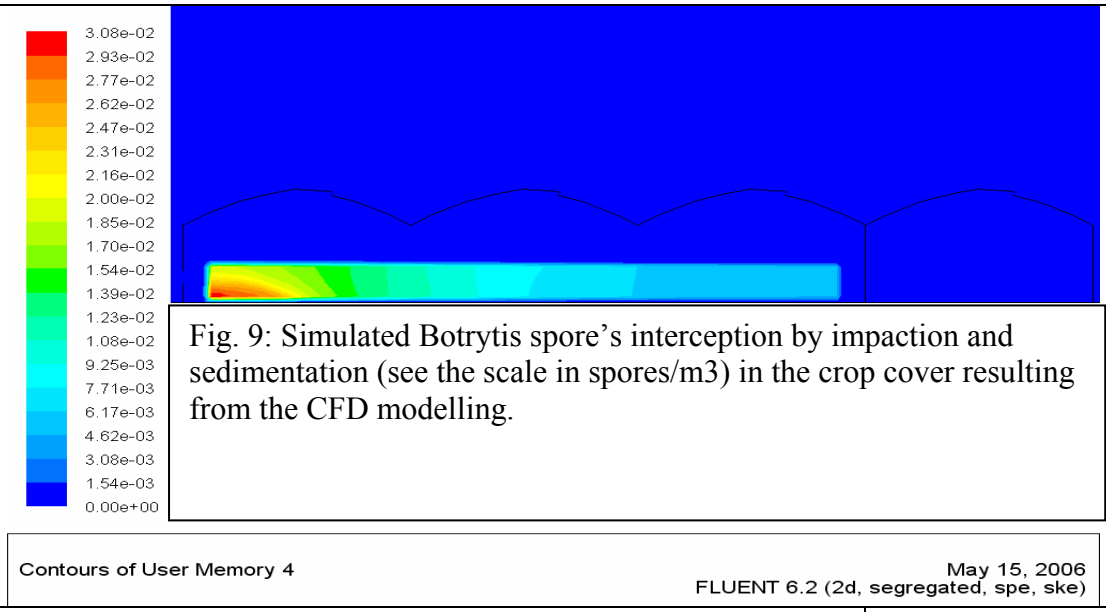


Fig. 9: Simulated Botrytis spore's interception by impaction and sedimentation (see the scale in spores/m3) in the crop cover resulting from the CFD modelling.

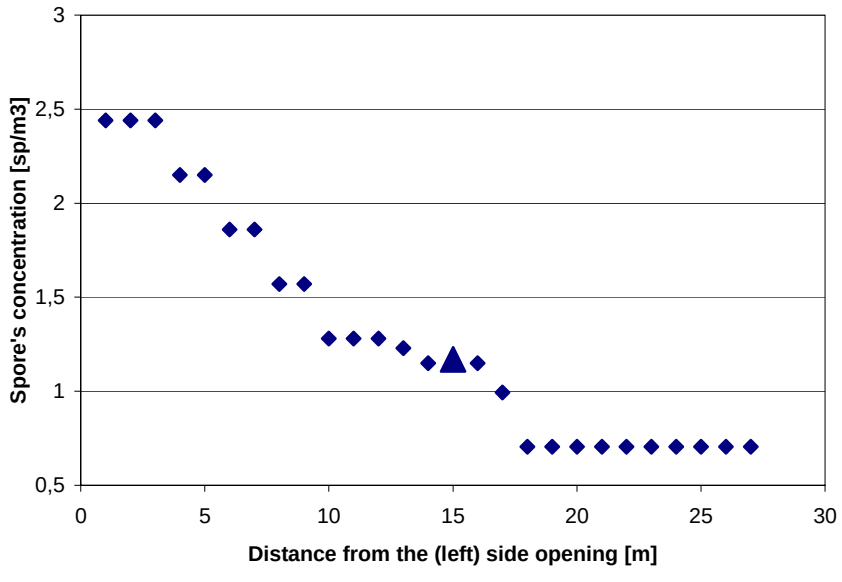


Fig. 10: Comparison of inside spore's concentration measured in the middle of the greenhouse and the simulated transverse distribution of spore's concentration resulting from the CFD modelling.