

## MODELLING *BOTRYTIS CINEREA* SPORE EXCHANGES AND PRODUCTION IN UNHEATED GREENHOUSES

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### SUMMARY

In order to devise optimal and sustainable strategies to protect greenhouse grown crops against *Botrytis cinerea*, production of spores inside the greenhouses and exchanges with the outside need to be monitored. Such knowledge could be used for adequate timing of measures aimed at limiting the entry of exogenous inoculum and others aimed at limiting the multiplication of the endogenous inoculum. In an earlier study, a model based on spore mass balance was developed to estimate the production of *B. cinerea* spores inside a greenhouse and the number of spores entering and leaving the greenhouse. This model was successfully used in a heated glasshouse. As much of the tomato production also occurs in more open and less controlled conditions, the present study aimed at testing the model in non heated plastic greenhouses (tunnels) where environmental parameters known to influence spore exchanges and production differ from a glasshouse setting. When diseased plants were present in the tunnel, the production of spores over 24 h estimated by the model was equivalent to 5 to 10% of the maximum potential amount present on the plants, which was coherent with the amount of spores likely to be released by the action of wind on sporulating lesions. Exchange of spores was toward the outside. When the tunnel was empty or contained disease-free plants, fewer spores were exchanged and the spore movement was globally from the outside toward the inside of the tunnel.

*Key words:* conidia quantification, air sampling, airborne pathogen, integrated pest management.

### INTRODUCTION

*Botrytis cinerea* Pers. Fr. can attack more than 200 plant species. This fungus is one of the most damaging pathogens of protected crops including tomato and lettuce. On tomato, infections generally occur on pruning

wounds and result in the development of stem cankers. They can girdle the stem and lead to premature death of the plant (Nicot and Baille, 1996). Fruits can also be attacked and rendered unmarketable. In greenhouse lettuce, *B. cinerea* develops at the heart of the heads or on the older leaves where humidity is the highest. The development of soft rot on those leaves requires extra trimming at harvest, which reduces yield and may compromise marketability of the heads, depending on the severity of the attack. When conditions are favourable abundant sporulation occurs on diseased plants (Nicot *et al.*, 1996). The spores are easily disseminated by wind and can move with air currents to neighbouring crops (Jarvis, 1962; Harrison and Lowe, 1987).

Chemical control is possible but *B. cinerea* presents a high risk of developing fungicide resistance because of its high genetic variability (Giraud *et al.*, 1997; Beever and Weeds, 2004). Indeed, phenotypes showing resistance to synthetic fungicides with different biochemical modes of action have been found in various crops (Besri and Diatta, 1985; Johnson *et al.*, 1994; Yourman and Jeffers, 1999; Leroux *et al.*, 2002). Moreover, chemical control becomes less acceptable from an environmental and sociological point of view. In order to progress towards sustainable farming, a more rational use of chemicals is needed. Thus, a better understanding of epidemics and the development of tools for risk assessment are necessary for the rational reduction of fungicide use and the implementation of integrated crop protection.

The origin of the inoculum that induces epidemics in tomato and lettuce greenhouses is poorly known. Such knowledge would be useful for greenhouse management. If the inoculum originates mostly from the outside of the greenhouse, a rationalized management of ventilation could be used to limit inoculum entry and reduce disease outbreak. If the inoculum is produced predominantly inside the greenhouse (on diseased plants or on dead organic substrates), climate regulation and crop treatments could be useful to avoid disease development. To develop efficient integrated protection against airborne fungi and sustainable plant production, it is then necessary to know whether spores enter from the outside or are produced inside the greenhouse.

Direct measurement of fluxes and production of fun-

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gal spores in a greenhouse is a difficult task and modelling may provide a useful alternative. Boulard *et al.* (2008) developed a model which estimates spore production in a greenhouse. The basis for the model is that the amount of inoculum present in the air of a greenhouse (measured by the inside concentration of air-borne inoculum) depends on spore production (on diseased plants or on dead organic matter), but it is affected by deposition (on the plants, floor and structures) and by the fluxes of inoculum between the inside and the outside, which in turn is dependant on the outside concentration of air-borne inoculum and on the air exchange rate through openings (Fig. 1). This model was used to study a heated glasshouse composed of several compartments (Boulard *et al.*, 2008), a type of structure which represents approximately one third of the surface of sheltered crops in France. The most common type of greenhouses, used over nearly 4,500 ha (Agreste, 2005), are plastic tunnels which are more open and have smaller volumes than glasshouses. They are generally not or only minimally heated and the climate inside is less regulated than in glasshouses.

The aim of our study was to determine if the model developed for a glasshouse was relevant for use in tunnels. Specifically, our purpose was to ascertain whether: (i) the values estimated by the model were influenced by the presence or absence of spore-bearing plants in the tunnel and (ii) inoculum production calculated with the model was coherent with that estimated from the amount of spores present on the plants. To this end, an evaluation phase was first carried out in an empty tunnel and in a tunnel that contained inoculated plants bearing a known number of sporulating lesions. In the second phase, the model was used in tunnels containing tomato or lettuce crops.

## MATERIAL AND METHODS

**Experimental set up.** Experiments were carried out in INRA's experimental tunnels at Avignon (43° 55'N, 4°48'E). The tunnels (8×16 m) were oriented north to south. Passive ventilation resulted from lateral side vents (1.2 mm×12 mm) equipped with wind-deflecting nets to prevent damage from strong winds. The four central tunnels comprised 6 rows of 30 tomato plants (cvs Swansson and De Ruiters), with a density of 1.4 plants m<sup>-2</sup>. A lettuce crop was grown in the two other tunnels (cvs Faustina and Rijk Zwaan) with a density of 12 plants m<sup>-2</sup>. Tomatoes were drip-irrigated and lettuces were irrigated by sprinkling according to common commercial practice. The side vents of tomato tunnels were automatically opened when the inside temperature increased beyond 23°C. The vents of lettuce tunnels were kept opened, with a maximum opening area of 28.8 m<sup>2</sup> (representing 12% of the total cover

surface), because of high summer temperatures.

### Estimating inoculum production inside the tunnels.

Using the model of Boulard *et al.* (2008) the spore balance was computed during replicate series of 24 h periods. Spore production was calculated with the formula:

$$Pi\Delta t = V_s\Delta Ci + Di\Delta t - G(Co-Ci)\Delta t$$

where  $Pi$  represented the amount of airborne inoculum produced inside the tunnel per unit of time,  $\Delta t$  represents the 24 h time span used in our replicate experiments,  $V_s$  represents the total volume of air (in m<sup>3</sup>) in the tunnel,  $\Delta Ci$  represents the change in concentration of air-borne inoculum inside the tunnel during the 24 h period (in spores m<sup>-3</sup>),  $Di$  represents the rate of sedimentation of air-borne inoculum (in spores per unit of time),  $G$  represents the air exchange rate between the inside and the outside and  $Co$  represents the concentration of air-borne inoculum outside of the tunnel (in spores m<sup>-3</sup>). Thus, the terms of the formula  $Pi\Delta t$  represents the amounts of inoculum produced during the 24 h time span,  $V_s\Delta Ci$  represents the change in spore content inside the total volume  $V_s$  of the tunnel,  $Di\Delta t$  represents the amount of inoculum removed from the air by sedimentation during the 24 h period and  $G(Co-Ci)\Delta t$  represents the net balance of inoculum exchange between the inside and the outside of the tunnel during the 24 h period.

**Estimation of the air exchange rate between the inside and the outside of the tunnel.** According to Boulard and Baille (1995), the air exchange rate  $G$  (m<sup>3</sup>h<sup>-1</sup>) can be estimated as:

$$G = \frac{So}{2} Al \sqrt{2g \frac{(To - Ti) Hc}{To} + (C_w U^2)}$$

where  $So$  is the opened surface of the tunnel (m<sup>2</sup>),  $Al$  is the discharge coefficient of the openings,  $C_w$  is the wind effect coefficient,  $g$  is the gravity constant (9.81 m sec<sup>-2</sup>),  $Ti$  and  $To$  are the air temperature inside and outside of the tunnel,  $Hc$  is the equivalent chimney height (m) and  $U$  is the wind speed (m sec<sup>-1</sup>) outside of the tunnel.

In tomato tunnels, where vents opening was adjusted according to inside temperature, the opened surface  $So$  was monitored continuously with the help of potentiometers fixed on the rolling axes of the vents. The data were collected every 5 seconds by a CR10X data logger (Campbell Scientific, UK) and averaged over 15 min periods. To estimate  $Al$  and  $C_w$ , we used a gas-tracing technique (N<sub>2</sub>O decay rate) as described by Fatnassi *et al.* (2002). Two cases were distinguished, as the estimates may be affected by crop height, depending on the wind speed outside the tunnels (Fatnassi *et al.*, 2009). When wind speed was below 1 m sec<sup>-1</sup> values of  $Al$  and  $C_w$  were estimated as 0.74 and 0.068, respectively, and

used for the calculation of  $G$  regardless of crop height. For wind speeds over  $1 \text{ m sec}^{-1}$ , the term

$$\sqrt{2g \frac{(T_o - T_i) Hc}{T_o} \frac{Hc}{2}}$$

is negligible and the calculation of  $G$  can be simplified

$$\text{as } G = \frac{S_o}{2} Al\sqrt{C_w} U.$$

In this case the overall wind effect coefficient on ventilation ( $Al\sqrt{C_w}$ ) was shown in a preliminary work to be highly correlated with the angle between wind direction and the vents (Fatnassi *et al.*, 2009). On the basis of that work, we used the formulas presented in Table 1 for our estimations of  $Al\sqrt{C_w}$ .

**Table 1.** Calculation of the overall wind effect coefficient on ventilation ( $Al\sqrt{C_w}$  when wind speed is over  $1 \text{ m} \times \text{sec}^{-1}$  (from Fatnassi *et al.*, 2009).

Vegetation height	$Al\sqrt{C_w}$
20 cm	$= 0.258[\cos(\theta)]^2 - 0.0065\cos(\theta) + 0.31$
1 m	$= 0.1604[\cos(\theta)]^2 + 0.0006\cos(\theta) + 0.2369$
2.13 m (to the roof)	$= 0.0278[\cos(\theta)]^2 - 0.0021\cos(\theta) + 0.2$

$\theta$  is the angle between wind vector and vents' surface.

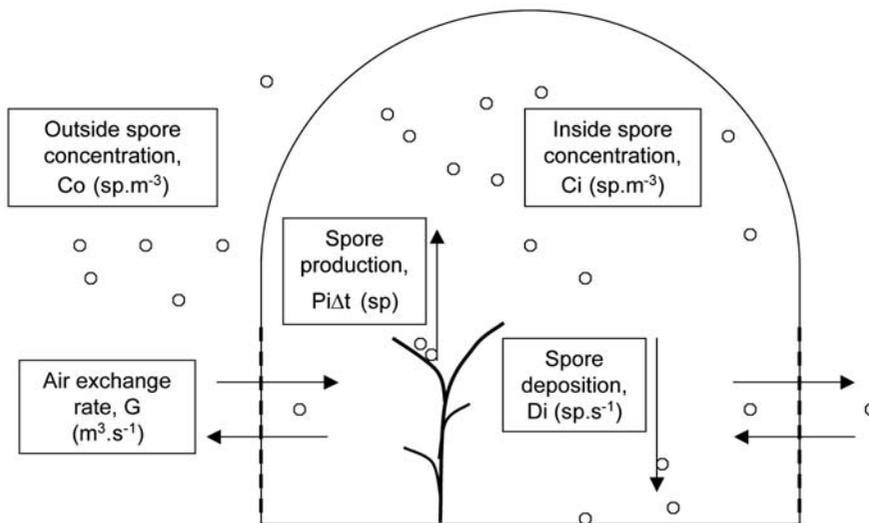
**Climatic data.** Air temperature and relative humidity were monitored continuously inside and outside the tunnels, using ventilated HMPC35C probes (Vaisala, Finland). Wind speed and wind direction were meas-

ured by a wind monitor (model 05103, Young, USA) attached to a mast installed on the roof of one of the tunnels. All data were collected every 5 sec. by a CR10X data logger and averaged over 15 min periods.

**Biological measurements.** Inside ( $C_i$ ) and outside ( $C_o$ ) concentrations of air-borne inoculum were assessed with two high throughput jet samplers (Burkard Manufacturing, UK) with a flow rate of  $0.75 \text{ m}^3 \text{ min}^{-1}$ . Petri plates (9 cm diameter) containing 20 ml of a *Botrytis*-selective medium were placed at the base of the sampler's settling chamber. We used the *Botrytis* Spore Trap Medium (BSTM) of Edwards and Seddon (2001), amended with a high amount of agar (2.5%) to limit dehydration of the medium during hot periods. Sampling was carried out during 24 h periods. In each sampling day, three Petri plates were placed successively in each sampler at 8:00, 12:00 and 17:00 (central European time).

Sedimentation of air-borne inoculum was assessed with 12 deposition plates homogeneously distributed in the tunnel. The plates contained 20 ml of BSTM medium and were placed 20 cm above the ground. The plates were exposed for 24 h. When the weather was hot and dry, 5 ml of sterile distilled water were added on the surface of the medium to avoid dehydration.

After exposure, all Petri plates (either from Burkard samplers or used as deposition plates) were incubated in the laboratory at  $20^\circ\text{C}$  and were examined daily for a fortnight for fungal development. As several species with initially similar morphology may grow on BSTM medium, every colony showing radially-branched hyphae and producing a dark brown halo in the medium was transferred to potato dextrose agar (PDA) for further identification. After 10 days of incubation on PDA, when typical *Botrytis* sporulation appeared on the



**Fig. 1.** Terms of a spore balance model in a tunnel with two side vents. Spores (represented by small circles) can move between the inside and the outside of the tunnel through the open vents. *B. cinerea* spores are likely to be produced by diseased plants and death organic debris on the soil.

plates, the number of *Botrytis* colony forming units (CFU) was recorded. These counts were then used to estimate the density of air-borne inoculum per cubic meter of air (Burkard samplers) and the numbers of air-borne inoculum units deposited per second per square meter of tunnel (deposition plates). To facilitate reading, the term “air-borne inoculum unit” will be simplified to “spore” in the rest of this paper, although it cannot be ruled out that some of the air-borne inoculum quantified in our experiments consisted of groups of spores or other forms, such as mycelial fragments embedded in small debris particles.

**Evaluation tests in absence of crops.** Two experiments were conducted outside the cropping season (one in autumn and one in spring). For both experiments the model was first tested in an empty tunnel for 5 consecutive days, in conditions representing an expected lack of inoculum production. In a second step a batch of 15 potted tomato plants, all bearing sporulating lesions of *B. cinerea*, was introduced in the tunnel and exposed for 6 days. In a third step, only carried out for spring testing, a new batch of 30 plants with sporulating lesions was introduced and exposed for an additional 7 days. All the plants had been inoculated and incubated in a climatic chamber at 21°C seven days before their introduction in the tunnel. In the second test, the amount of spores present on the plants was determined. On the basis of earlier work (Nicot *et al.*, 1996; Fruit, 2001), it was estimated that each of the plants of the first batch during the spring experiment carried on average 53,000 spores at the time of introduction in the greenhouse, while each of those of the second batch carried *ca* 12,000 spores. These data were used for comparison with spore production values provided by the model.

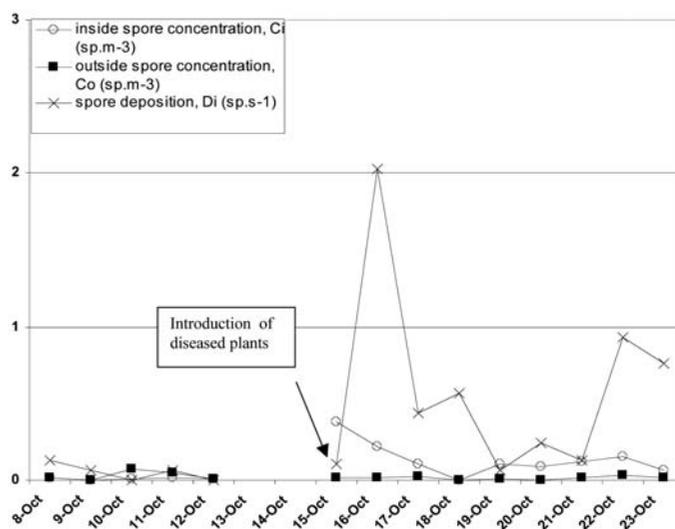
**Use of the model in tunnels with crops.** During the 2007 growing season, trials were conducted at crop level in two tunnels. The model was used to estimate spore production in a tomato tunnel at three different crop stages with plant height varying from 20 cm to 2.10 m (from April to June), and was used in a lettuce tunnel (May). During the growing season tomato and lettuce crops were monitored weekly for incidence of grey mould and other possible pests. Every symptom on tomato plants and every rotten lettuce with typical sporulation were recorded. Disease incidence was calculated by estimating the number of plants attacked by *B. cinerea* out of the total number of plants in the tunnel.

**Statistical analyses.** Statistical analyses were done with StatView (version 5, SAS Institute). Statistical inferences were made at the 5% level of significance, unless indicated otherwise. Non parametric tests were used to determine correlations (Spearman test) and significant differences (Mann and Whitney) between data of different days.

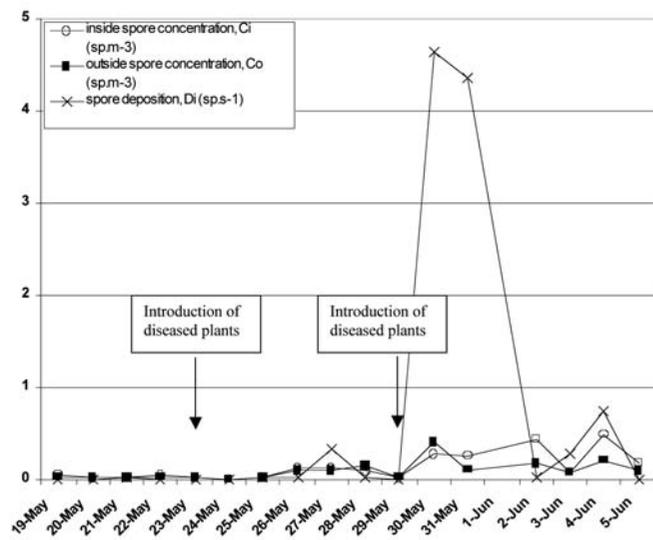
## RESULTS AND DISCUSSION

**Evaluation of the model in absence of crops.** To evaluate the relevance of the model for the estimation of spore production inside a tunnel, we compared the values computed with the model in a situation representing lack of inoculum production (experiment in a empty tunnel) and in a situation in which a known amount of inoculum was present on plants introduced in the tunnel. Overall, the values provided by the model coincided well with the experimental situation, with some level of day-to-day variability as detailed below.

In these two experiments, spore trap counts provided low values of inside and outside air-borne inoculum concentration (Fig. 2 and 3) before the introduction of diseased potted plants in the tunnel. During the days when the tunnel was empty, there was no statistically significant difference between inside and outside concentrations (Mann and Whitney,  $P=0.30$  for the first experiment and  $P=0.24$  for the second one). On average, inoculum deposition amounted to 0.052 and 0.01 spores per second on the total surface of the tunnel, for the autumn and the spring experiment, respectively, which corresponded to 35 and 7 spores deposited per square meter during a 24 h period. In the autumn experiment, the estimated spore exchange [ $G(C_o-C_i)\Delta t$ ] was low but positive during the days when the tunnel was empty, indicating that more spores were entering than leaving the tunnel (Fig. 4). For the same situation in the spring experiment, the estimated spore exchange rate was also low, but it was negative, suggesting that

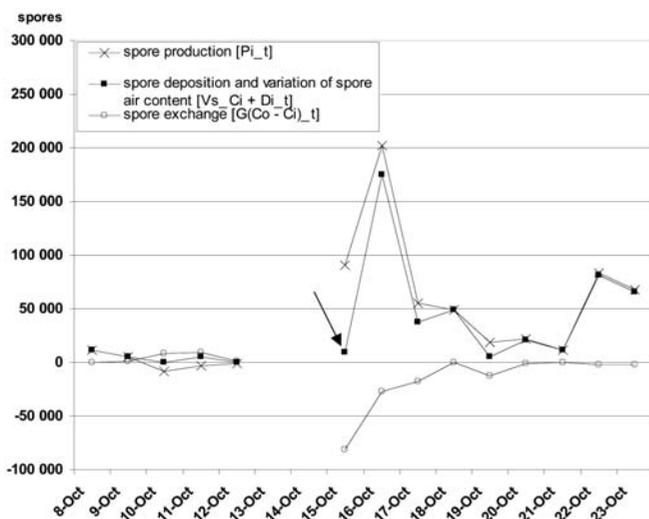


**Fig. 2.** Inside and outside spore concentration and spore deposition during the autumn evaluation test. On October 15, a batch of 15 plants was introduced in the tunnel (biological data were not recorded on October 12 and 13).  $C_o$  and  $C_i$  were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler.  $D_i$  was assessed with the help of deposition plates containing a semi-selective medium.



**Fig. 3.** Inside and outside spore concentration and spore deposition during the spring evaluation test. On May 23 a first batch of 15 diseased plants was introduced in the tunnel. It was replaced on May 29 by a second batch of 30 plants.  $C_o$  and  $C_i$  were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler.  $D_i$  was assessed with the help of deposition plates containing a semi-selective medium.

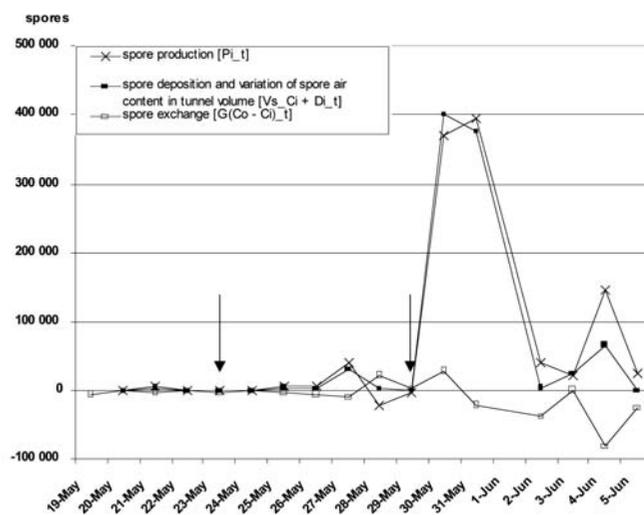
more spores left than entered the tunnel (Fig. 5). The estimations of daily spore production provided by the



**Fig. 4.** Variations of the terms of the spore balance in the autumn validation test. On October 15, a batch of 15 diseased plants was introduced in the tunnel (indicated by the arrow).  $C_o$  and  $C_i$  were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler.  $D_i$  was assessed with the help of deposition plates containing a semi-selective medium.  $V_s$  represented the total volume of air (in  $m^3$ ) in the tunnel.  $G$  was calculated on the basis of the physical characteristics of the tunnel and climatic data (see M&M section). Spore production was calculated as  $P_i\Delta t = V\Delta C_i + D_i \Delta t - G(C_o - C_i) \Delta t$ .

model in the empty tunnels ranged from  $-8,397$  to  $+11,290$  spores in the autumn experiment and from 0 to 5,450 spores in the spring experiment. Negative values occurred mostly when inside and outside spore concentration values were very low and when outside concentration was higher than inside concentration or when spore deposition was low.

The effect of the introduction of spore-bearing plants in the tunnel on the estimates provided by the model was different between the two experiments. In the autumn experiment, both inside concentration and inoculum deposition increased as soon as diseased tomato plants were introduced in the tunnel (Fig. 2). The inside concentration was significantly greater than the outside concentration (Mann and Whitney,  $P=0.01$ ) and a maximum inoculum deposition of  $2.02$  spores  $sec^{-1}$  was recorded, which corresponded to  $1,366$  spores  $m^{-2} day^{-1}$ . The term representing the exchange of spores with the outside was null or negative, indicating that more spores were leaving than entering the tunnel (Fig. 4). One day after the introduction of spore-bearing plants, the estimate of spore production by the model reached  $201,900$  spores  $day^{-1}$ . Estimated spore production then decreased during the five following days but remained higher than the production estimated in the empty tunnel. Then another peak of  $82,955$  spores was observed on October 22. Globally, during this first validation test, the model indicated spore immigration into the tunnel



**Fig. 5.** Variations of the terms of the spore balance in the spring validation test. On May 23 a first batch of 15 diseased plants was introduced in the tunnel. It was replaced on May 29 by a second batch of 30 diseased plants (introduction indicated by arrows).  $C_o$  and  $C_i$  were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler.  $D_i$  was assessed with the help of deposition plates containing a semi-selective medium.  $V_s$  represented the total volume of air (in  $m^3$ ) in the tunnel.  $G$  was calculated on the basis of the physical characteristics of the tunnel and climatic data (see M&M section). Spore production was calculated as  $P_i\Delta t = V\Delta C_i + D_i \Delta t - G(C_o - C_i) \Delta t$ .

**Table 2.** Mean (minimum and maximum in brackets) of main components of the *Botrytis cinerea* spores balance in three trials performed in tunnels with tomato and lettuce crop.

Estimated crop height	Tomato trial 1 27 March-06 April		Tomato trial 2 23 April-04 May		Tomato trial 3 5 June-14 June		Lettuce trial 21-31 May	
	20 cm	1 meter	1 meter	2 meters	2 meters	2 meters	10 cm	10 cm
Outside spore concentration, Co (sporesxm <sup>-3</sup> ) <sup>v</sup>	0.019 (0; 0.04)	0.018 (0.002; 0.04)	0.018 (0.002; 0.04)	0	0	0	0.007 (0; 0.03)	0.007 (0; 0.03)
Inside spore concentration, Ci (sporesxm <sup>-3</sup> ) <sup>v</sup>	0.014 (0; 0.04)	0.009 (0.004; 0.01)	0.009 (0.004; 0.01)	0.0015 (0; 0.008)	0.0015 (0; 0.008)	0.0015 (0; 0.008)	0.03 (0.002; 0.17)	0.03 (0.002; 0.17)
Spore deposition, Di (sporesxs <sup>-1</sup> ) <sup>w</sup>	0.014 (0; 0.05)	0.06 (0; 0.17)	0.06 (0; 0.17)	2.19E-05 (0; 1.2 E-04)	2.19E-05 (0; 1.2 E-04)	2.19E-05 (0; 1.2 E-04)	0.74 (0; 2.69)	0.74 (0; 2.69)
Air exchange rate, G (m <sup>3</sup> xs <sup>-1</sup> ) <sup>x</sup>	0.6 (0; 2.64)	1.32 (0; 2.1)	1.32 (0; 2.1)	1.81 (1.1; 2.2)	1.81 (1.1; 2.2)	1.81 (1.1; 2.2)	4.54 (2.41; 10.67)	4.54 (2.41; 10.67)
Spore exchange (spores) <sup>y</sup>	-52 (-2,473; 1,870)	225 (-1,034; 2,328)	225 (-1,034; 2,328)	-246 (-1,224; 0)	-246 (-1,224; 0)	-246 (-1,224; 0)	-8,246 (-51,566; 1,014)	-8,246 (-51,566; 1,014)
Daily spore production estimated by the model, PiΔt (spores) <sup>z</sup>	1,308 (-1,090; 4,227)	5,396 (-2,328; 16,277)	5,396 (-2,328; 16,277)	2,900 (0; 15,513)	2,900 (0; 15,513)	2,900 (0; 15,513)	78,778 (531; 284,346)	78,778 (531; 284,346)

v: Co and Ci were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler. w: Di was assessed with the help of deposition plates containing a semi-selective medium. x: G was calculated on the basis of the physical characteristics of the tunnel and climatic data (see M&M section). y: spore exchange was calculated as  $G(\text{Co}-\text{Ci})$ . z: spore production was calculated as  $\text{Pi}\Delta t = \text{V}\Delta\text{Ci} + \text{Di}\Delta t - \text{G}(\text{Co}-\text{Ci})\Delta t$ .

in absence of diseased plants and spore emigration from the tunnel in the presence of substantial amounts of spores on diseased plants.

In the spring experiment, the introduction of a first batch of 15 spore-bearing plants (May 23) had no obvious incidence on spore balance (Fig. 3). During the six days following this introduction there was no statistically significant difference between inside and outside spore concentrations (Mann and Whitney,  $P=0.90$ ). Observed inoculum deposition remained low (at the most  $0.35 \text{ spore sec}^{-1}$  which corresponded to  $235 \text{ spores m}^{-2} \text{ day}^{-1}$ ). However, the exchange of spores with the outside was negative during most of the time, indicating that spores were leaving the tunnel (Fig. 5). The estimation of spore production provided by the model was between  $-20,500$  and  $+39,400 \text{ spores day}^{-1}$ . In comparison, the potential spore production estimated by measuring stem lesions on the plants was *ca*  $8 \times 10^5$  spores. The maximum daily production calculated with the model was thus equivalent to 5% of the estimated number of spores present on the plants.

The replacement of the first 15 plants by a new batch of 30 diseased plants (May 29) was followed by an increase in air-borne inoculum concentrations (Fig. 3). There was no significant difference between inside and outside concentrations during the 7 days when the second batch was in the tunnel (Mann and Whitney,  $P=0.20$ ). When deposition reached a maximum of  $4.6 \text{ sp sec}^{-1}$  on the tunnel ground, 3,130 spores were deposited on each square meter in 24 h. Globally, after the second introduction of plants, the model indicated that there was a flux of spores from the inside of the tunnel toward the outside (Fig. 5). Up to 81,000 spores moved from the inside to the outside of the tunnel in 24 h (June 4). The estimation of spore production per day reached a maximum of 396,000. At the time when the new diseased plants were introduced in the tunnel, they carried an estimated total amount of at least  $3.7 \times 10^6$  spores. The production calculated with the model was thus equivalent to 10% of the estimated potential spore amount present on diseased plants.

To explain these results we could hypothesize that the release of spores from diseased plants may have been only partial. For fungi such as *B. cinerea*, that lack an active mechanism of expulsion for conidia, passive release of spores necessitates the occurrence of wind gusts (Aylor, 1990). Using a wind tunnel, Harrison and Lowe (1987) showed that only 6.2% of the total spores of *B. cinerea* present on diseased bean leaves could be removed by ten series of 20 sec wind gusts with velocities increasing from 1 to  $10 \text{ m sec}^{-1}$ . In support of our hypothesis, the peaks of spore production measured by the model during the second test corresponded to peaks of wind speed. Peaks of wind speed occurred on May 30 ( $3.5 \text{ m sec}^{-1}$  for 3 consecutive quarters of an hour) and on June 4 (above  $1 \text{ m sec}^{-1}$  during 90% of the time).

**Use of the model in tomato and lettuce tunnels.** To verify if the model was applicable to commercial tunnels, it was tested four times in real-scale tunnels containing either tomato or lettuce crops.

During the three trials conducted from March to June in a tomato tunnel no epidemic development of grey mould was observed. Inside and outside spore concentration, deposition and estimated spore production were much lower than in the evaluation experiments (Table 2). There was no significant difference between inside and outside concentrations with P values of 0.36, 0.34, and 0.68, respectively, for the 3 trials. Deposition and inside and outside concentrations were particularly low in the third trial. The absence of viable air-borne inoculum might be due to important solar radiation during several bright sunny days, reaching  $10^3 \text{ W m}^{-2}$  at midday (national platform CLIMINRA, INRA, Avignon, France). It has been shown that a one-day exposure to sunny solar radiation ( $5 \times 10^3 \text{ W m}^{-2}$ ) decreases the viability of *B. cinerea* spores below 5% and a two-day exposure reduces it to zero (Rotem and Aust, 1993). The spore exchange was very variable during the three trials, changing from negative to positive from one day to the next with small values, indicating that the flux of spores between inside and outside the tunnel was changing a lot. The mean spore production calculated with the model was low, not exceeding 17,000 spores in 24 h (Table 2).

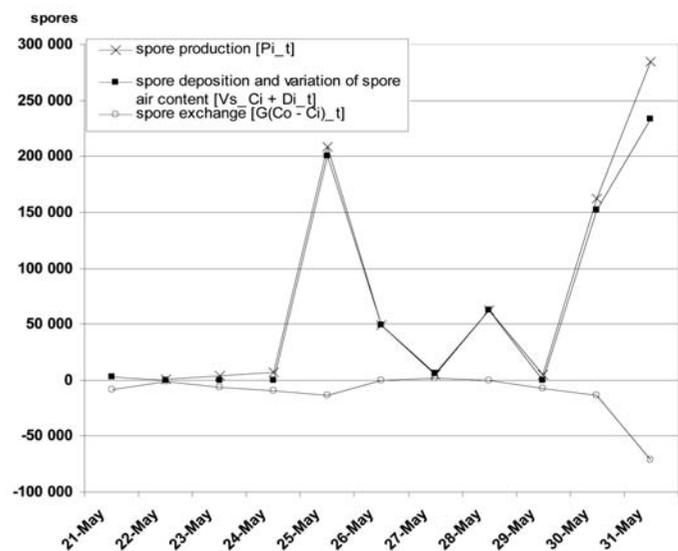
In the lettuce tunnel, *B. cinerea* symptoms started to develop in the beginning of May. During the period when the spore fluxes were modeled in this tunnel, average grey mould incidence was 3.7%. Grey mould was often associated with *Sclerotinia* symptoms. *B. cinerea* sporulation took place on the oldest leaves, near the collar of the plants. Biological data such as inside concentration and deposition were higher than in the tomato tunnel where no sporulating lesions were observed (Table 2). Deposition showed two peaks (2.3 sp sec<sup>-1</sup> and 2.6 sp sec<sup>-1</sup> respectively) and reached a maximum of 224 640 spores settling down on the total surface of the tunnel during 24 h ( $1,755 \text{ sp m}^{-2} \text{ day}^{-1}$ ). Surprisingly, inside spore concentration and deposition were not significantly correlated ( $R_{\text{Spearman}} = 0.403$ ,  $P = 0.2021$ ). Outside spore concentration remained low. There was no significant difference between inside and outside spore concentration (Mann and Whitney,  $P = 0.0738$ ). The mean air exchange rate, G with a value of  $4.54 \text{ m}^3 \text{ sec}^{-1}$ , was higher than in the two evaluation experiments and the three tomato trials. This high value was due to the fact that the vents remained wide open during the whole lettuce trial. As a consequence, the total volume of air in the tunnel was renewed every 69 seconds in average. During 10 of the 11 days when inoculum was monitored in the lettuce trial, the term of spore exchange was null or negative, indicating that there was a flux of spores from the inside towards the outside of the tunnel (Fig. 6). The spore production estimated by the

model showed two peaks of 208,000 (May 25) and 284,000 (May 31) spores produced in 24 h.

Inside and outside spore concentrations recorded in the present study (Table 2) are lower than those measured by Boulard *et al.* (2008) in a rose glasshouse. In this study, the reported range of inside spore concentration was 0.8-0.9 sp m<sup>-3</sup> on average and that for outside spore concentration was 0.8-3.0 sp m<sup>-3</sup>. This difference may be due either to differences in environmental conditions and to the little incidence of grey mould in our crops compared to the rose crop, or to possible differences in the methods used for air sampling.

A striking result of our work is that in all trials (but particularly in the absence of diseased plants) the spore production estimated by the model was sometimes negative. These negative values could suggest a high death rate of existing inoculum on certain days. They could also point to a limit of this type of model in conditions of low production of endogenous inoculum. The same reservations may apply when positive spore production was estimated in the absence of visible disease symptoms, although an alternative hypothesis could be that undetected inoculum production occurred on debris, soil-borne sclerotia or alternative hosts.

The objective of this study was to determine if the spore balance model developed by Boulard *et al.* (2008) could be used in unheated tunnels to estimate fluxes of inoculum and endogenous spore production by an air-



**Fig. 6.** Variations of the terms of the spore balance in the lettuce tunnel during the course of experiment (21-31 May). Co and Ci were assessed by collecting airborne inoculum on a semi-specific medium using a high throughput air sampler. Di was assessed with the help of deposition plates containing a semi-selective medium. Vs represented the total volume of air (in m<sup>3</sup>) in the tunnel. G was calculated on the basis of the physical characteristics of the tunnel and climatic data (see M&M section). Spore production was calculated as  $Pi\Delta t = V\Delta Ci + Di \Delta t - G(Co - Ci) \Delta t$ .

borne plant pathogen. When diseased plants carrying large amounts of spores were introduced in the tunnel, the estimate of spore production provided by the model was coherent with the amount of spores likely to be released by the action of wind on sporulating lesions. The production estimated in such cases represented 5 to 10% of the total maximum amount available on diseased plants for air-borne dissemination. However, incoherent estimations of spore production were sometimes obtained, in the form of negative values unlikely to reflect a realistic level of spore mortality inside the tunnel. This occurred mostly when inside and outside spore concentration values were very low and when outside concentration was higher than inside concentration, or when spore deposition was very low. A more precise measure of these values could perhaps avoid the inconsistent estimations. Thus, improvement of model utilization could result from improving spore quantification. A possible way to achieve this would be to combine spore trapping methods with the use of real-time PCR (Suarez *et al.*, 2005) in place of the microbiological isolation method used in the present study. It could allow rapid detection and quantification of *B. cinerea* inoculum present in the air inside and outside the greenhouse. The ultimate step to set up optimal and sustainable protection strategy against *B. cinerea* will be the determination of relation between air-borne spore concentration and risk of disease spread in the greenhouse.

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