European Journal of Plant Pathology (2005) 113:377–394
DOI 10.1007/s10658-005-4269-y

Quantifying and modelling the mobilisation of inoculum from diseased leaves and infected defoliated tissues in epidemics of angular leaf spot of bean

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Accepted 5 October 2005

Key words: corrected basic rate of infection, daily multiplication factor, defoliation, epidemic threshold, *Phaeoisariopsis griseola*, progeny/parent ratio

Abstract

Daily multiplication factor (number of daughter lesions per mother lesion per day) values were experimentally measured in four replications of a monocyclic experiment on angular leaf spot (ALS) of bean, where sources of inoculum were artificially established within a bean canopy, on the ground (defoliated infected leaves), or both. Daily multiplication factor of lesions in the canopy (DMFRc) was higher than that of infectious, defoliated tissues (DMFRd) in all replications. Both DMFRc and DMFRd were strongly reduced under dry compared to rainy conditions. Under rainy conditions for spore dispersal DMFRd was about two to three times smaller than DMFRc. Defoliated leaves may nevertheless represent a significant source of infection, depending on the amount of infectious tissues. Mother lesions within the canopy generated more daughter lesions in the medium (or lower) layers of the canopy than at its upper level (DMFRc higher at the medium and lower layers of a canopy), whereas DMFRd values seemed to decrease with height in the canopy. A mechanistic simulation model that combines host growth and disease-induced defoliation was designed to simulate the respective contributions of the two components of the dual inoculum source of a diseased canopy (infected foliage and defoliated infectious tissues), and varying infectious periods in both sources. Simulations suggest that higher DMFRc values have a large polycyclic effect on epidemics whereas that of DMFRd is small, and that large effects of the infectious period of lesions in the canopy are found when DMFRc is high. Simulations using experimentally measured DMFRc and DMFRd values indicated much stronger epidemics in rainy compared to dry conditions for spore dispersal, but disease persistence in the latter. The implications of considering a dual source of inoculum in the course of a polycyclic process are discussed with respect to epidemic thresholds.

Introduction

Host plant defoliation, which is caused by a number of foliar pathogens, has attracted a long-standing interest among botanical epidemiologists (Kushalappa and Ludwig, 1982; Waggoner and Berger, 1987; Johnson, 1987; Thal and Campbell, 1988; Bergamin Filho et al., 1997; Carneiro et al., 2000). This is because disease-induced defoliation, while being one important injury mechanism leading to yield loss, may also strongly affect the course of an epidemic via a number of factors and feed-backs, including the availability of healthy tissues, changes in the physical environment of disease, and host growth itself. Disease-induced defoliation also has a direct effect on disease

measurements themselves, as it affects the amount of disease that can be observed on the canopy (Kushalappa and Ludwig, 1982; Thal and Campbell, 1988; Nelson and Campbell, 1993), and this has led to several attempts to correct, or account, for defoliation.

While defoliated, infected, tissues are removed from the epidemiological process when biotrophic pathogens are involved, this is not the case with many necrotrophic, or hemi-necrotrophic foliar pathogens (Thal and Campbell, 1988; Duthie and Campbell, 1991). In diseases caused by such pathogens, defoliated, infected, and infectious tissues may become another source of inoculum. Plant stands that are affected by diseases caused by such defoliating pathogens are thus exposed to a dual source of inoculum: propagules that are produced on lesions on the standing canopy and those produced by defoliated tissues. The processes by which this dual source influences the course of an epidemic then becomes an interesting research issue of its own, which may be addressed by considering the respective mobilisation of the two types of propagules, and by quantifying the progeny of lesions on attached or detached leaves, i.e., the daily multiplication factors (Zadoks, 1971) that refer to the two kinds of sources.

The purpose of this work was twofold: (1) to generate experimental measurements of daily multiplication factors of infectious lesions on attached and detached tissues in the bean-*Phaeoisariopsis griseola* pathosystem, and (2) to simulate the dynamics of an infected canopy where a dual source of inoculum exists, in order to compare the respective contributions of the two sources and analyse the epidemiological outcomes of empirical measurements of daily multiplication factors in this pathosystem.

Material and methods

Experimental design

An experiment was designed in order to measure the daily multiplication factors of inoculum, represented by sporulating angular leaf spot (ALS) lesions located either on attached or defoliated bean leaves. The experiment was replicated four times from April to June 2004, at the EMBRAPA Centre Arroz e Feijão, Goiania, Brazil (Lat. 16°28′0′, long. 49°17′0′, elevation 823 m).

Experimental micro-plots consisted of plastic trays (40×40 cm) which contained 15 cm of soil from the EMBRAPA Centre experimental fields. Plants of the cultivar Rosinha-G2 were seeded with 10 cm spacing (four rows of four hills, with one seeding per hill). There were three treatments corresponding to three categories of inoculum sources, and a control where no source was established to quantify external inflows of inoculum. The first treatment consisted of establishing infected plants in pots at the centre of the micro-plots to mimic infected leaves in the canopy (Treatment 1, T1). The second treatment consisted of placing infected leaves on the ground to mimic infected defoliated leaves (Treatment 2, T2). The last treatment consisted of both establishing potted infected plants at the centre of the micro-plots and placing infected leaves on the ground (Treatment 3, T3). This last treatment corresponds to actual conditions, where infected leaves both in the canopy and defoliated are present in a diseased bean stand.

Observations before establishment of the inoculum sources

Three leaf layers were considered in the micro-plots, bottom (BT), mid-height (MH), and upper (UP) layers of the canopy (Plaut and Berger, 1980). These leaf layers corresponded to three classes of height: BT, 0–20 cm; MH, 20–40 cm; UP, >40 cm. Each leaf of each plant was tagged with three different colours corresponding to the three leaf layers.

Immediately before establishing the inoculum sources, the number of leaves per leaf layer (BT, MH and UP) was counted in each micro-plot, and the leaf area of each layer was estimated from a sample of four plants per micro-plot. The length and width of the central leaflet of each leaf were measured, and leaf areas (cm^2) were estimated using the relationship $y = 1.52\ x - 4.54$, where x is the product (length $\times$ width) of the central leaflet and y the foliar area of the leaf. This equation was derived from regression analysis ($R^2 = 0.97$), on an independent data set of 33 leaves. No leaf layer of upper canopy (UP) was observed in treatment T3 of the replication REP4.

Establishment of the inoculum sources

The isolate of *Phaeoisariopsis griseola* used for the inoculation of the sources was isolate '410.5' collected on cultivar LM932044323 at the EMBRAPA Centre. Inoculation procedure used was as described by Sartorato (2004). The first trifoliate leaf of each potted plant was inoculated 15 days after sowing, with a spore suspension of 20×10^4 spores ml^{-1} concentration. Inoculated plants were incubated for 40 h in a humid chamber (RH > 95%) at 25 ± 2 °C, with a 12 h photoperiod. Plants were transferred to greenhouse benches after incubation.

Plants of the experimental micro-plots were transferred to outdoor conditions 1 month after sowing, on the ground, with 5m spacing, prior to establishing the different inoculum sources. Number of lesions, severity (Godoy et al., 1997), and leaf area of the infected source plants were assessed prior to their establishment in the experimental micro-plots. Total number of lesions per source plant ranged from 674 to 946 for treatments T1 and T2, and 1538 to 1645 for treatments T3 of the different replications. After 24 h of incubation into the humid chamber to favour sporulation, the inoculum sources were established into the micro-plots for four consecutive days. Two pots containing two infected plants each were placed in treatments T1 and T3 (four infected leaves). Infected leaves were located in the leaf layer MH (20–40 cm). In treatments T2 and T3, leaves of four infected plants were cut and placed on the ground (i.e., four infected leaves per micro-plot). After the 4-day period, all inoculum sources were removed from all micro-plots, which were transferred into the humid chamber for 32 h to favour infection. The micro-plots were then transferred to greenhouse benches.

The number of lesions per leaflet was assessed on each leaf of each micro-plot plant 12 days later. The total number of lesions per plot was corrected by subtracting the number of lesions observed in the control plot (without source inoculum). Numbers of lesions corrected for the control were converted to lesion densities (number of lesions per leaf cm^2). Data were analysed using a split plot linear mixed model using the procedure PROC MIXED of SAS (Garrett et al., 2004). Treatment (T1, T2, or T3) was considered as fixed effect, main unit, leaf layer (BT, MH, or UP) as fixed effect, sub-unit, and replication (REP1, REP2, REP3, or REP4) as random effect. Square-root transformation was used to stabilise variances.

Weather data (rainfall, wind speed, relative humidity and radiation) during the 4 days of plant source exposition were recorded at the meteorological station of the EMBRAPA Centre, within a few hundred meters from the experimental area.

Calculation of the daily multiplication factors from experimental data

The daily multiplication factor (DMFR) of a lesion is defined as the number of daughter lesions per mother lesion per day (dimension: $[N_{\text{lesion}}\ N_{\text{lesion}}{}^{-1}\ \text{day}^{-1}]$; Zadoks, 1971; Zadoks and Schein, 1979). Both lesions located on attached leaves and on defoliated leaves may contribute to new infection of healthy sites. The daily multiplication factor may thus be considered in two terms, DMFRc and DMFRd, which refer to lesions attached to the canopy, and to defoliated leaves, respectively. These parameters can be calculated from the experimental data for the different leaf layers as

$$\text{DMFRc} = (\text{LN}/\text{LNc})/\tau$$

$$\text{DMFRd} = (\text{LN}/\text{LNd})/\tau$$

where LN is the lesion number observed on each leaf layer (BT, MH, or UP); LNc and LNd, are the lesion numbers present on the inoculum sources located on attached leaves (treatment T1) and located on defoliated leaves (treatment T2), respectively, and τ is the duration of exposure of healthy sites to the inoculum source(s), i.e., 4 days. Daily multiplication factors DMFRc and DMFRd were also calculated for the entire canopy by accumulating the numbers of lesions observed in each leaf layer, BT, MH, and UP, and dividing by the number of lesions in the inoculum source(s) and by duration τ.

Model structure

The system under study is a 1 m^2 bean crop infected by ALS. The model we used elaborates on the mechanistic systems model by Zadoks (1971), with a 1-day time step for integration. Two processes were added to this initial structure, host growth and defoliation (Figure 1). State variables

are sites which represent a canopy surface unit, which may be vacant, latent, infectious, or removed from the epidemiological process. Propagules can be deposited on the canopy and infect vacant (healthy) sites. The model also includes state variables accounting for defoliated, infectious, and defoliated, removed, sites. Two kinds of state variables are distinguished, depending on whether these are attached to a given residence time (latency and infectious periods), or not. If a residence time is to be considered, these are modelled as box-car trains. This model structure was implemented for dynamic simulation using the STELLA® Research Version 6 for PC and the STELLA® II Version 4 for Macintosh (High Performance Systems, 1996). Table 1 lists the different state variables, rates and driving variables of the model.

State variables and flows

There are seven categories of sites that change state over time (Table 1). Healthy sites (HSITE) on a bean canopy increase in number with a rate of growth (RGROW). When infected by a spore, they become latent (LAT), with a rate of infection (RINF). Latent sites (LAT) become infectious (INFc) with a rate of appearance (RAPP) that is determined by the latent period (p). The latent period is defined as the delay between host infection by a spore and the beginning of sporulation (Zadoks and Schein, 1979). Infectious sites located on canopy leaves (INFc) may undergo a defoliation process caused by the disease, with a rate of defoliation (RDEFi), and become infectious sites on defoliated leaves (INFd). Defoliation of infectious sites simultaneously leads to defoliation of healthy sites (HSITE) with a rate of defoliation (RDEFh), and become healthy defoliated sites (HDEF). The structure of the model considers two infectious periods, either on canopy leaves, ic, or on defoliated leaves, id. The infectious period is defined as the delay between the beginning and the end of sporulation (Zadoks and Schein, 1979). After this time delay, the infectious sites on canopy leaves (INFc) and on defoliated leaves (INFd) are removed (REMc and REMd) with a rate of

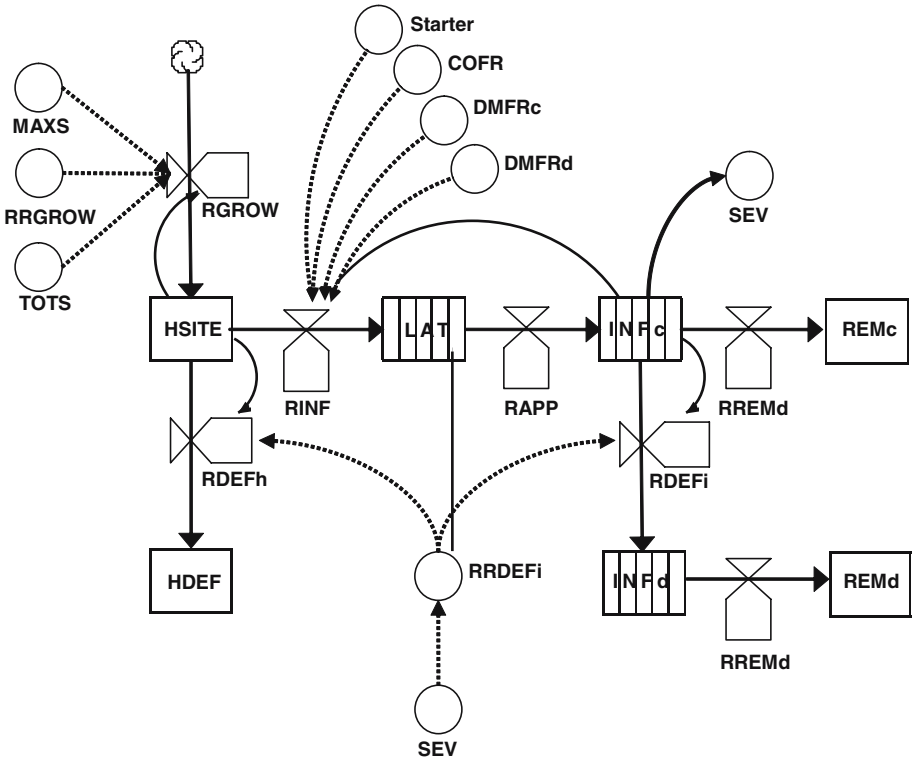

Figure 1. Model structure integrating the defoliation of the infectious sites located on diseased canopy leaves. Symbols of state variables, rates and driving variables are listed in Table 1.

Table 1. List of state variables, rates and driving variables

	Meaning of symbol	Dimension
State variables		
HSITE	Number of healthy sites	[N_{sites}]
HDEF	Number of healthy sites on defoliated leaves	[N_{sites}]
INFc	Number of infectious sites on canopy leaves	[N_{sites}]
INFd	Number of infectious sites on defoliated leaves	[N_{sites}]
LAT	Number of latent sites	[N_{sites}]
REMc	Number of removed infectious sites on canopy leaves	[N_{sites}]
REMd	Number of removed infectious sites on defoliated leaves	[N_{sites}]
Rates		
RAPP	Rate of appearance of infectious sites on canopy leaves	[N_{sites} day^{-1}]
RDEFh	Rate of defoliation of healthy sites	[N_{sites} day^{-1}]
RDEFi	Rate of defoliation of infectious sites	[N_{sites} day^{-1}]
RGROW	Rate of growth of healthy sites	[N_{sites} day^{-1}]
RINF	Rate of infection of healthy sites	[N_{sites} day^{-1}]
RRDEFi	Relative rate of defoliation of infectious sites	[N_{sites} N_{site}^{-1} day^{-1}]
RREMc	Rate of removal of infectious sites on canopy leaves	[N_{sites} day^{-1}]
RREMd	Rate of removal of infectious sites on defoliated leaves	[N_{sites} day^{-1}]
RRGROW	Relative rate of growth of healthy sites	[N_{sites} N_{site}^{-1} day^{-1}]
Driving variables		
COFR	Correction factor	[N_{sites} N_{site}^{-1}]
DMFRc	Daily multiplication factor of infectious sites on canopy leaves	[N_{sites} N_{site}^{-1} day^{-1}]
DMFRd	Daily multiplication factor of infectious sites on defoliated leaves	[N_{sites} N_{site}^{-1} day^{-1}]
MAXS	Maximal number of sites	[N_{sites}]
SEV	Severity	[–]
Starter	Starter of the incoming of primary inoculum	[–]
TOTS	Total number of sites	[N_{sites}]

removal (RREMc and RREMd). These rates are functions of the infectious periods, ic and id, respectively. Following Zadoks (1971), passage of sites through these periods (p, ic and id) is simulated using box-car trains.

Equation for canopy growth

The rate of healthy site growth (RGROW) for the different leaf layers and for the entire bean canopy can be described according to a logistic growth:

$$\mathrm{RGROW} = \mathrm{RRGROW} \times \mathrm{HSITE} \times (1 - (\mathrm{TOTS}/\mathrm{MAXS}))$$

where RRGROW is the relative rate of growth, HSITE is the number of healthy sites, TOTS is the total number of sites of the system, and MAXS is the maximum, accumulated number of sites which can be produced, i.e. a carrying capacity. Table 2 lists the values for RRGROW and MAXS. The maximum leaf area index (LAI) of the entire canopy was estimated as 5 approximately 55 days after sowing (for cv. Rosinha) (Bergamin Filho et al., 1997). The size of the sites was set to 10^{-5} m^2, which represents the average size of an ALS lesion (Bergamin Filho et al., 1997; Allorent,

Table 2. Parameters values used for simulations performed for different leaf layers and an entire bean canopy

Leaf layer[a]	HSITE[b]	Starter[c]	HGROWI[d]	RRGROW[e]	MAXS[f]
BT	1000	200	1	0.35	2×10^5
MH	1000	71	15	0.35	2.5×10^5
UP	1000	4	25	0.35	0.5×10^5
Overall	1000	300	1	0.25	5×10^5

[a]BT, bottom, MH, mid-height, and UP, upper leaf layers; OV, overall (refers to estimates on the entire canopy: leaf layers BT, MH, and UP).
[b]Initial number of healthy sites [N_{sites}].
[c]Initial primary inoculum [N_{sites}].
[d]Host growth initiation in days after sowing [days].
[e]Relative rate of growth of healthy sites during host growth [N_{sites} N_{site}^{-1} day^{-1}].
[f]Maximal number of sites [N_{sites}].

unpublished data). MAXS for the entire canopy was thus set to a value of 5×10^5 sites (Table 2). The maximum LAI was estimated as 2, 2.5 and 0.5 for the leaf layer BT, MH, and UP, respectively, corresponding to MAXS values of 2×10^5, 2.5×10^5, and 0.5×10^5 (Table 2). Growth of the different leaf layers was shifted over time (Lieth and Reynolds, 1988). The dates of initiation of growth for the different leaf layers and entire bean canopy are listed in Table 2. These initiation dates and maximum LAI values correspond to simulating a progressive halt in growth at approximately at 30, 45, and 55 days after sowing, respectively, for leaf layers BT, MH, and UP.

Equation for canopy infection

The rate of infection of healthy sites (RINF) can be written as

$$\mathrm{RINF} = \mathrm{COFR} \times ((\mathrm{DMFRc} \times \mathrm{INFc}) + (\mathrm{DMFRd} \times \mathrm{INFd})) + \mathrm{starter}$$

where COFR is the correction factor (Zadoks, 1971) corresponding to the term (1−x) (Van der Plank, 1963) for disease increase; and DMFRc and DMFRd are the daily multiplication factors for infectious sites on canopy (INFc) or defoliated leaves (INFd). The 'starter' term initiates epidemics by incorporating an initial primary inoculum. Epidemics of ALS of bean are usually observed relatively late in the crop cycle (Saettler, 1991; Allen et al., 1998; Allorent and Savary, submitted); 'starter' was thus assumed to initiate epidemics at $t=30$ days after sowing (das). The different values of primary inoculum (STARTER) according to the leaf layers BT, MH, and UP, and entire canopy are listed in Table 2. They are calculated as a proportion of 1 initial site for 1000 healthy sites at time 30 das. This proportion corresponds to one initial site per leaf (with a maximal leaf area of 100 cm^2 for cv. Carioca) (Carneiro et al., 2000). At time 31 das, 1/1000 healthy sites become latent. The correction factor is calculated as the proportion of healthy sites on the total number of sites located on the canopy, and can be written as:

$$\mathrm{COFR} = \mathrm{HSITE}/(\mathrm{HSITE} + \mathrm{LAT} + \mathrm{INFc} + \mathrm{REMc})$$

Equation for defoliation

The defoliation of infectious sites leads to the removal of healthy sites, as both infectious and healthy sites may belong to the same diseased leaves (Savary and Servat, 1991).

The rate of defoliation of infectious sites on canopy leaves (RDEFi) is a function of a relative rate of defoliation (RRDEFi), which itself depends on disease severity (SEV). A negative exponential model was fitted to data ($R^2=0.96$) using data from Willocquet et al. (2004) to relate these two later parameters as:

$$\mathrm{RRDEFi} = 0.2205 \times [1 - \exp(-18.56 \times \mathrm{SEV})]$$

where severity is the ratio of infectious to the total number of sites:

$$\mathrm{SEV} = \mathrm{INFc}/(\mathrm{HSITE} + \mathrm{LAT} + \mathrm{INFc} + \mathrm{REMc})$$

The rate of defoliation of infectious sites (RDEFi) is the product of the relative rate of defoliation (RRDEFi) by the number of infectious sites (INFc). Conversely, the rate of defoliation of healthy sites (RDEFh) is the product of the relative rate of defoliation (RRDEFi) by the number of healthy sites (HSITE).

Underlying hypotheses

The model structure and equations as described in the previous subsections describe nine important hypotheses:

1. Infectious defoliated sites contribute to new infections.
2. DMFRc and DMFRd are held constant during simulation runs.
3. No defoliation is incurred by physiological leaf senescence.
4. Defoliation is triggered only by disease severity, which determines a relative rate of defoliation of both healthy and infectious sites.
5. Defoliation acts on infectious and healthy sites in the canopy, but not on latent sites.
6. Healthy sites cannot be infected when defoliated.
7. There are two infectious periods, ic and id, corresponding to infectious sites in the canopy

and infectious sites on defoliated tissues, respectively.
8. No lesion expansion is considered.
9. No disease aggregation is considered.

Model verification and sensitivity analysis

Model verification aims at checking that hypotheses and underlying assumptions of the model are properly handled by the simulation programme (Teng, 1981). As the structure of the model derives from an already tested, earlier systems model (Zadoks, 1971), which had been used in several other simulation models (e.g. Savary et al., 1990; Zadoks and Kampmeijer 1977), model verification concentrated on the specific new features of the model. These features are: (1) the existence of two infection sources (lesions within an infected canopy, and infectious tissues on defoliated leaves on the ground), and (2) the existence of leaf tissues that are still infectious after their shedding on the ground, for a given infectious period, which may numerically interact with the infectious period of attached (non-defoliated) lesions. Verification of the model therefore concentrated on these two groups of characteristics, by varying DMFRc and DMFRd simultaneously and independently (proper accounting of the two sources of inoculum), and by varying the infectious periods of attached and shed tissues. Three values of DMFRc and DMFRd (0.05, 0.25, 0.5), along with three two values of ic and id (5, 10 and 20 days) were used in combinations. Ranges selected for DMRc, DMRd, ic and id, were chosen to correspond to commonly used ranges in the literature (Van der Plank, 1963; Zadoks, 1971; Campbell and Madden, 1990; Savary et al., 1990; Lannou et al., 1994). Variations of number of sites and severity were studied as responses to these different combinations. Model verification runs were conducted with an epidemic onset (input of a proportion of 1 latent site per 1000 healthy sites) at 30 days after sowing, with values of the parameters estimated for the entire canopy (Table 2), and with a fixed latency period of 15 days. A series of 81 simulations was conducted with variations of DMFRc, DMFRd, ic, and id, permuting three values for each of four variables, to assess the sensitivity of the model's output expressed as area under ALS severity progress curve.

Simulated ALS epidemics using experimentally measured DMFRc and DMFRd values

The effects of values for DMFRc and DMFRd that were calculated from experimental data on ALS epidemics were studied using the simulation model. Variations of area under severity progress curve (AUSPC) were studied as responses to the different combinations of daily multiplication factors DMFRc and DMFRd. DMFR values for leaf layers BT, MH, UP, and for the entire canopy, of each of the four replications were used to perform 16 simulations. Simulated epidemics were performed using values of the parameters listed in Table 2. Infectious periods (ic and id) of 20 days and a latent period duration of 15 days were used for all simulations.

Results

Outdoor measurements of efficiency of inoculum sources

There were important changes in weather pattern (Table 3) when the four replications of the experiment were conducted. These included an overall decrease in temperatures (daily maximum and minimum averages), a strong reduction in relative humidity (daily maximum and minimum averages), and an increase in global radiation. There were also differences in rainfall, the two first replications having been exposed to some rainfall (5.6 and 2.3 mm), while the two last replications were not exposed to any rainfall during the four-day exposure period. Conducting the experiment during these four time periods therefore led to very contrasting environments where the efficiency of the two types of sources to spread disease could be assessed.

Numbers of lesions observed on plants of the control micro-plots (without inoculum source) were 5, 88, 0 and 0 lesions for replications 1, 2, 3 and 4, respectively. External inoculum was negligible compared to numbers of lesions observed on plants of the T1, T2, and T3 micro-plots (from 0 to 5%). Lesion densities measured at different leaf layers are shown in Figure 2. Replication 1 (T1, lesions in the canopy, Figure 2a) showed large lesion densities at all three leaf layers when the inoculum source was located within the canopy.

Table 3. Weather data during the four days of source exposition for each replication of the experiment

Replication	Month	T_{max} [a]	T_{min} [a]	RH_{max} [b]	RH_{min} [b]	WS_{max} [c]	P_{tot} [d]	RAD_{tot} [e]
REP1	April	28.4	18.9	98.2	58.2	6.3	5.6	14.4
REP2	May	28.9	17.8	93.2	46.4	6.7	2.3	16.4
REP3	June	26.4	12.6	86.2	38.6	4.2	0.0	17.7
REP4	June	27.5	15.3	78.8	33.4	7.2	0.0	18.3

Entries are means over the four days of source exposition.
[a] T_{max} and T_{min}, maximal and minimal temperature (°C), respectively.
[b] RH_{max} and RH_{min}, maximal and minimal relative humidity (%), respectively.
[c] WS_{max}, maximal wind speed (m s^{-1}).
[d] P_{tot}, total precipitation (mm).
[e] RAD_{tot}, total solar radiation (MJ m^{-2} day^{-1}).

Lesion densities appeared smaller when the source of inoculum was located on the ground (T2, infected defoliated leaves, Figure 2a), and seemed to decline with increasing height in the canopy. When both sources of inoculum were combined (T3, lesions in the canopy and infected defoliated leaves, Figure 2a), higher lesion densities were observed, compared to individual (canopy or ground) sources. The pattern in lesion densities yielded slightly higher values in the second replication (Figure 2b). While lesion densities in T1 (source of inoculum in the canopy) and T3 (canopy or ground sources of

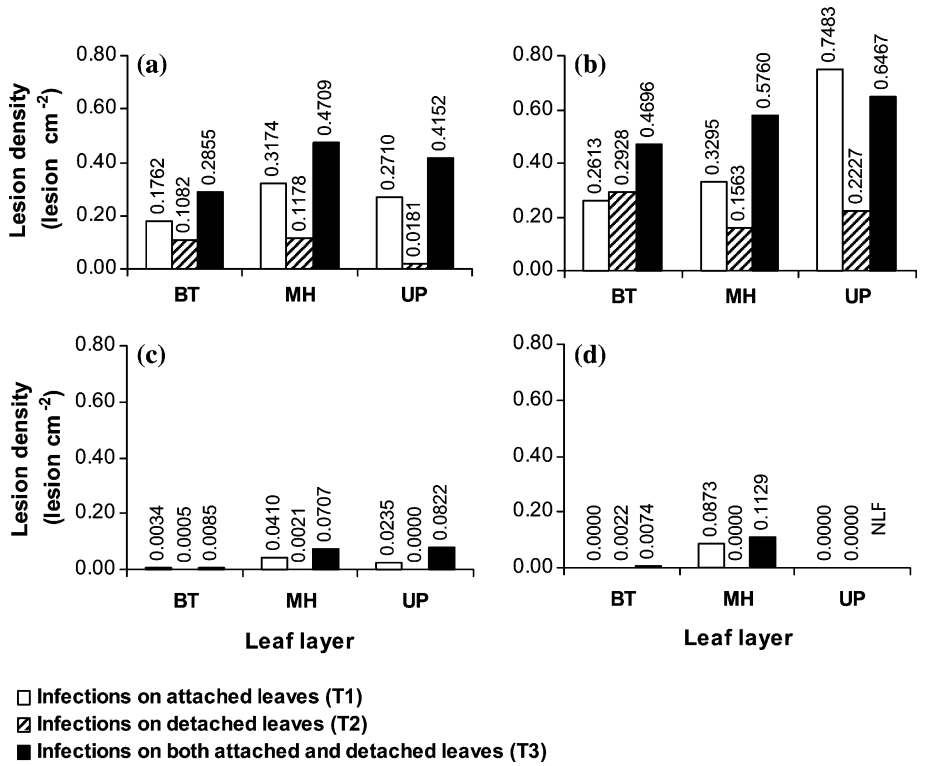

Figure 2. Lesion densities on the different leaf layers of the micro-plots according to the inoculum localization. Three treatments are considered with respect to localization of sporulating lesions: in the canopy (on attached leaves); on the ground (on detached leaves placed on the ground); or both. a–d correspond to replications REP1, REP2, REP3 and REP4, respectively. BT, MH, UP correspond to bottom, mid-height, and upper leaf layers, respectively. Each bar corresponds to one observation 12 days after source exposure in one replication. NLF, no leaf layer UP for treatment T3 in replication REP4 (d).

inoculum) increased with height in the canopy, the opposite trend was observed for treatment T2 (source of inoculum on the ground). In the third and fourth replications (Figures 2c and d), however, the observed lesion densities were approximately 10 times lower than in the first two replications. Data analysis using a mixed model indicated a significant ($P < 0.01$) inoculum positioning treatment effect and a significant ($P = 0.03$) leaf layer effect. A significant ($p = 0.04$) leaf layer by inoculum treatment interaction was also found, indicating that leaf layers did not respond to inoculum positioning in the same way. There were differences among treatments regarding this overall pattern; however while there were higher lesion densities in the middle height (MH) and upper (UP) layers in treatments 1 and 3, there was a regular decrease in densities from the bottom (BT), to the middle (MH) and to the upper (UP) layers in treatment 2 (inoculum on the ground) for replications 1 and 2 (Figures 2a and b). These differences in responses of leaf layers to inoculum positioning were reflected in the leaf layer by inoculum treatment interaction. It is important to note that few infections in treatment 2 were found in replications 3 and 4 (Figures 2c and d).

Experimental estimates of daily multiplication factor values

The outdoor experiments to measure the efficiency of sources to spread the disease led to very different daily multiplication values (Table 4). Estimates of the daily multiplication factor at the canopy level (DMFRc, Table 1) were twice to 60 times higher than estimates of the daily multiplication factor from ground inoculum (DMFRd). DMFRc was higher in the middle (MH) layer than in the upper (UP) or lower (BT) layers. There was also an overall decrease in values of DMFRc between the two first and the two last experiments, as indicated by the overall estimates (entire canopy) shifting from 0.829–0.989, to 0.045–0.055. Overall, therefore, the environment was less favourable to disease spread in experiments 3 and 4, as compared to experiments 1 and 2, with respect to inoculum available in the canopy. Experimental

Table 4. Daily multiplication factors (DMFRc and DMFRd) of the infectious sites calculated from experimental data (T1 and T2, respectively), and used for simulations performed for different leaf layers and an entire bean canopy

Replication	Leaf layer[a]	Experimental parameter values		Simulated epidemics[d]
		DMFRc[b]	DMFRd[c]	
REP1	UP	0.076	0.003	UP Replication 1
	MH	0.497	0.110	MH Replication 1
	BT	0.256	0.167	BT Replication 1
	Overall[a]	0.829	0.280	OV Replication 1
REP2	UP	0.176	0.028	UP Replication 2
	MH	0.542	0.346	MH Replication 2
	BT	0.271	0.116	BT Replication 2
	Overall[a]	0.989	0.490	OV Replication 2
REP3	UP	0.001	0.000	UP Replication 3
	MH	0.042	0.001	MH Replication 3
	BT	0.002	0.001	BT Replication 3
	Overall[a]	0.045	0.002	OV Replication 3
REP4	UP	0.000	0.000	UP Replication 4
	MH	0.055	0.000	MH Replication 4
	BT	0.000	0.001	BT Replication 4
	Overall[a]	0.055	0.001	OV Replication 4

[a]BT, bottom, MH, mid-height, and UP, upper leaf layers; OV, overall (refers to estimates on the entire canopy: leaf layers BT, MH, and UP)
[b]DMFRc, daily multiplication factor of infectious sites of canopy leaves
[c]DMFRd, daily multiplication factor of infectious sites of defoliated leaves
[d]See simulated outputs, Figure 6.

estimates of DMFRd differed strongly from those of DMFRc. In the first two replications, DMFRd values were highest in BT and MH layers, and lowest in UP layer (with at least a ten-fold reduction). DMFRd estimates in replications 3 and 4 were reduced by a factor of about 10^2, leading to very low values, and rendering comparisons across layers difficult. The results nevertheless indicated a very strong reduction of inoculum source efficiency with respect to ground-located inoculum (DMFRd) in the environmental conditions that prevailed in the third and fourth replications. These results (Table 4) therefore generate a wide array of experimentally measured values of both parameters, DMFRc and DMFRd, with contrasting variation of the two parameters (i.e., both being high, DMFRc high and DMFRd low, DMFRc low and DMFRd zero, and both parameters low), which are reflections of variation of the environment where spore dispersal prevailed. These values represent a useful set of environment-related parameter estimates to simulate epidemics.

Model verification

The behaviour of the model in terms of responses to varying values of DMFRc, DMFRd, ic and id is summarised in Figures 3 and 4. The response of the model is shown for a few sets of parameter combinations only, but for a number of simulated outputs: numbers of sites (infectious in the canopy, infectious and defoliated, removed in the canopy, removed and defoliated, and latent in the canopy, Figure 3), and number of healthy sites, number of defoliated sites, ALS severity (Figure 4).

A joint reduction in values of DMFRc and DMFRd from 0.50 to 0.25, and 0.05 resulted in a strong reduction of disease, as shown in Figures 3a, c, and f. A reduction (20 vs. 10 days) of the infectious periods ic and id (Figures 3a and b) resulted in a reduction of current, simulated (running) numbers of infectious sites (INFc and INFd), and in an increase in numbers of removed lesions (REMc and REMd) as sites remained infectious for a shorter period of time. A decrease in the running numbers of latents (LAT) was also simulated, as a result of each infectious lesion having a smaller progeny during its shortened infectious period. Reduction of DMFRc from 0.5 to 0.05 resulted in a reduction of latent and infectious periods (INFd and INFc) which was extremely strong (compare Figures 3a and d), whereas the same reduction in DMFRd had a much slighter impact on the numbers of infected (LAT, INFc, INFd) sites (compare Figures 3a and e). In all simulations, a larger running number of latent sites was simulated, compared to the numbers of infected (LAT, INFc, INFd) and post-infectious sites (REMc and REMd); on average, there were seven times more latents than any other categories. This reflected the relatively long latency period duration used in all runs (15 days).

Depending on parameter values, (e.g., Figure 3b) the number of infectious sites (INFc, INFd) may decrease over time. This is because they may become removed at the end of their infectious period.

Healthy sites declined progressively in numbers as severity increased, and defoliated tissues increased with severity (Figure 4). A reduction in both DMFRc and DMFRd values (Figures 4a, c and f) led to very strong reductions in disease severity, and a strong reduction in defoliation; the latter reduction was not as strong as the former, which is a reflection of the calculation of the rate of defoliation used in the model. As ic and id reduced from 20 to 10 days (Figures 4a and b), a reduction in severity was observed, leading to increased running numbers of healthy sites (HSITE), and decreased defoliation. This also led to fluctuating severity values, reflecting the counteracting effects of plant growth (site production, which depends on HSITE) and defoliation. Reduction of DMFRc from 0.5 to 0.05 resulted in a strong increase in healthy sites, and a strongly reduced defoliation and severity (compare Figures 4a and d), whereas the same reduction in DMFRd also resulted in increased HSITE, decreased HDEF, and decreased SEV, but to a much lesser extent (compare Figures 4a and e).

In simulation run A, it is worth noting that with DMFRc = DMFRd = 0.50; ic = id = 20 days (and a latency period of 15 days), that is a set of parameters that represent very favourable conditions for the epidemic throughout the season (except perhaps for the latency period), a maximum (terminal) severity of only 2% was simulated.

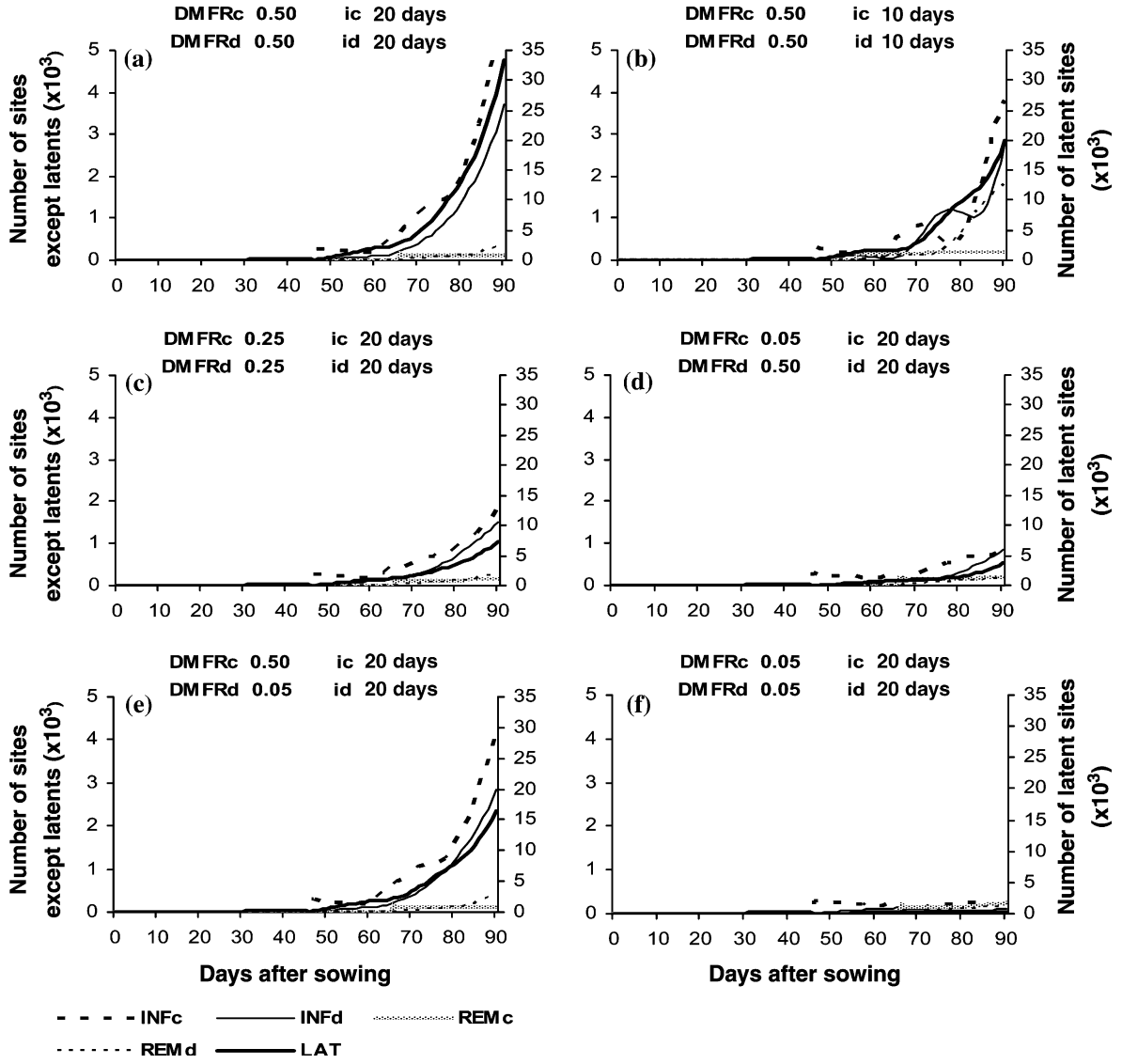

Figure 3. Simulations of variation in the numbers of sites in a diseased bean crop (in the canopy or on defoliated leaves): latent, infectious and removed sites, at different parameter values. DMFRc, daily multiplication factors of infectious sites on canopy leaves (Table 1), and DMFRd, daily multiplication factors of infectious sites on defoliated leaves; ic, infectious periods of infectious sites on canopy leaves, and id, infectious periods of infectious sites on defoliated leaves; LAT, latent sites, INFc, infectious sites on canopy leaves, INFd, infectious sites on defoliated leaves, REMc, removed sites on canopy leaves, REMd, removed sites on defoliated leaves. Numbers of initial healthy sites were 1000; a proportion of 1 latent site per 1000 healthy sites was introduced in the system at 30 days after sowing.

Sensitivity analysis

Simulated areas under ALS severity progress curves (AUSPC) corresponding to 81 combinations of three values of four parameters (ic, id, DMFRc, DMFRd) are shown in Figure 5. An overall increase of AUSPC with increasing ic and id is indicated. Increase in AUSPC with id was larger at high ic; the effect of high ic was larger than that of id. The increase in AUSPC was even stronger with high DMFRd and DMFRc. The latter parameter had by far the strongest effect of all four parameters. While the effect of increasing ic was small at low DMFRc,

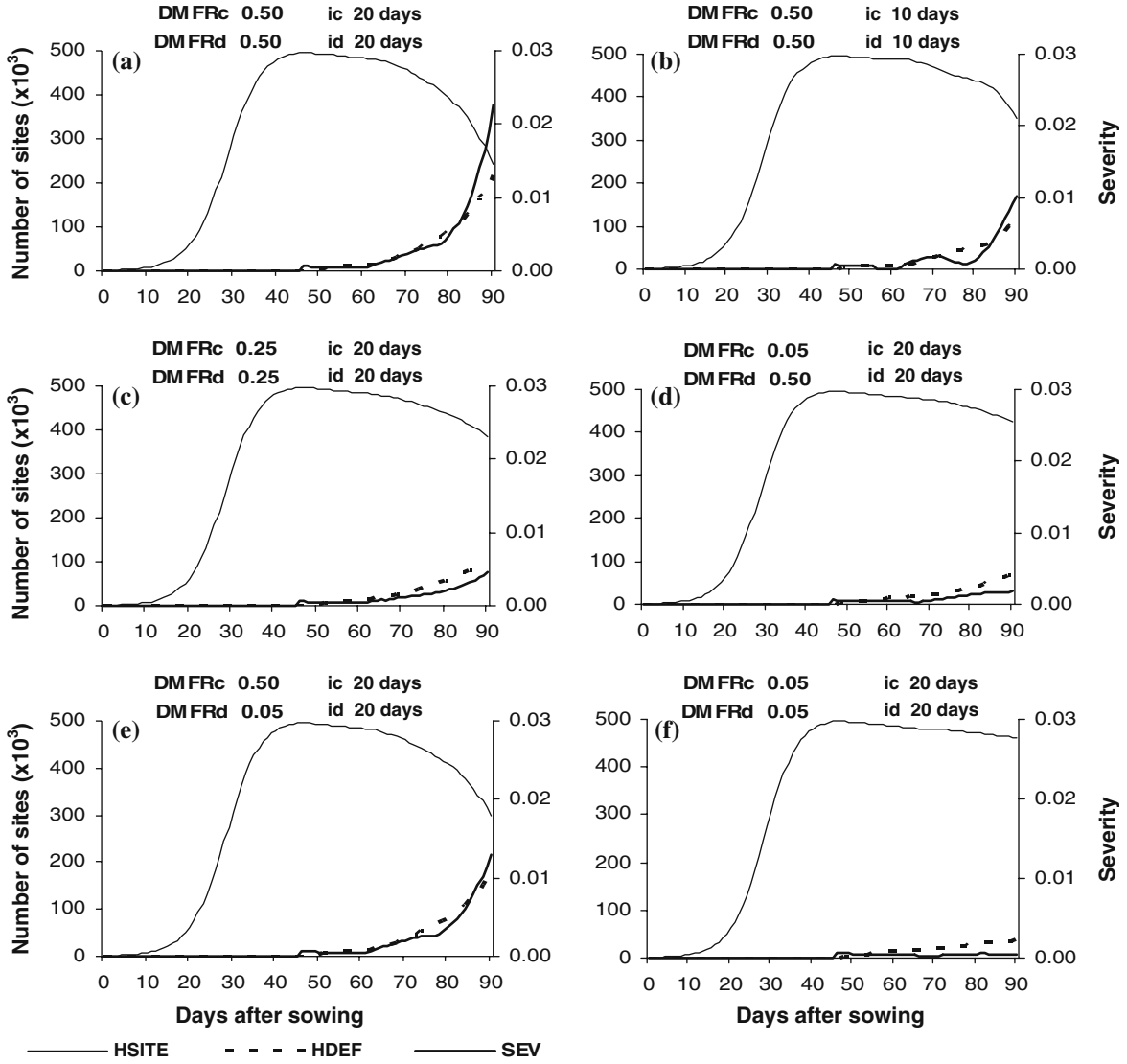

Figure 4. Simulations of variation in the numbers of healthy sites in a diseased bean crop (in the canopy or on defoliated leaves), and of severity at different parameter values. DMFRc, daily multiplication factors of infectious sites on canopy leaves (Table 1), and DMFRd, daily multiplication factors of infectious sites on defoliated leaves; ic, infectious periods of infectious sites on canopy leaves, and id, infectious periods of infectious sites on defoliated leaves; HSITE, healthy sites on canopy leaves, HDEF, healthy sites on defoliated leaves, SEV, severity on canopy leaves. Numbers of initial healthy sites were 1000; a proportion of 1 latent site per 1000 healthy sites was introduced in the system at 30 days after sowing.

it was much stronger at high DMFRc, indicating a substantial interaction between the two parameters.

Simulated ALS epidemics using experimentally measured DMFRc and DMFRd values

Simulated areas under ALS severity progress curves using experimental DMFRc and DMFRd values are shown in Figure 6, where each replication is represented by simulated outputs at the bottom (BT), mid-height (MH), and upper part (UP) of the canopy, and for the overall canopy (OV). AUSPC values were larger in the lower part of the canopy and decreased with height. Simulated AUSPC for the entire canopy (OV) were largest, and were larger than the accumulation of AUSPC values for individual layers, a reflection of the polyclic nature of

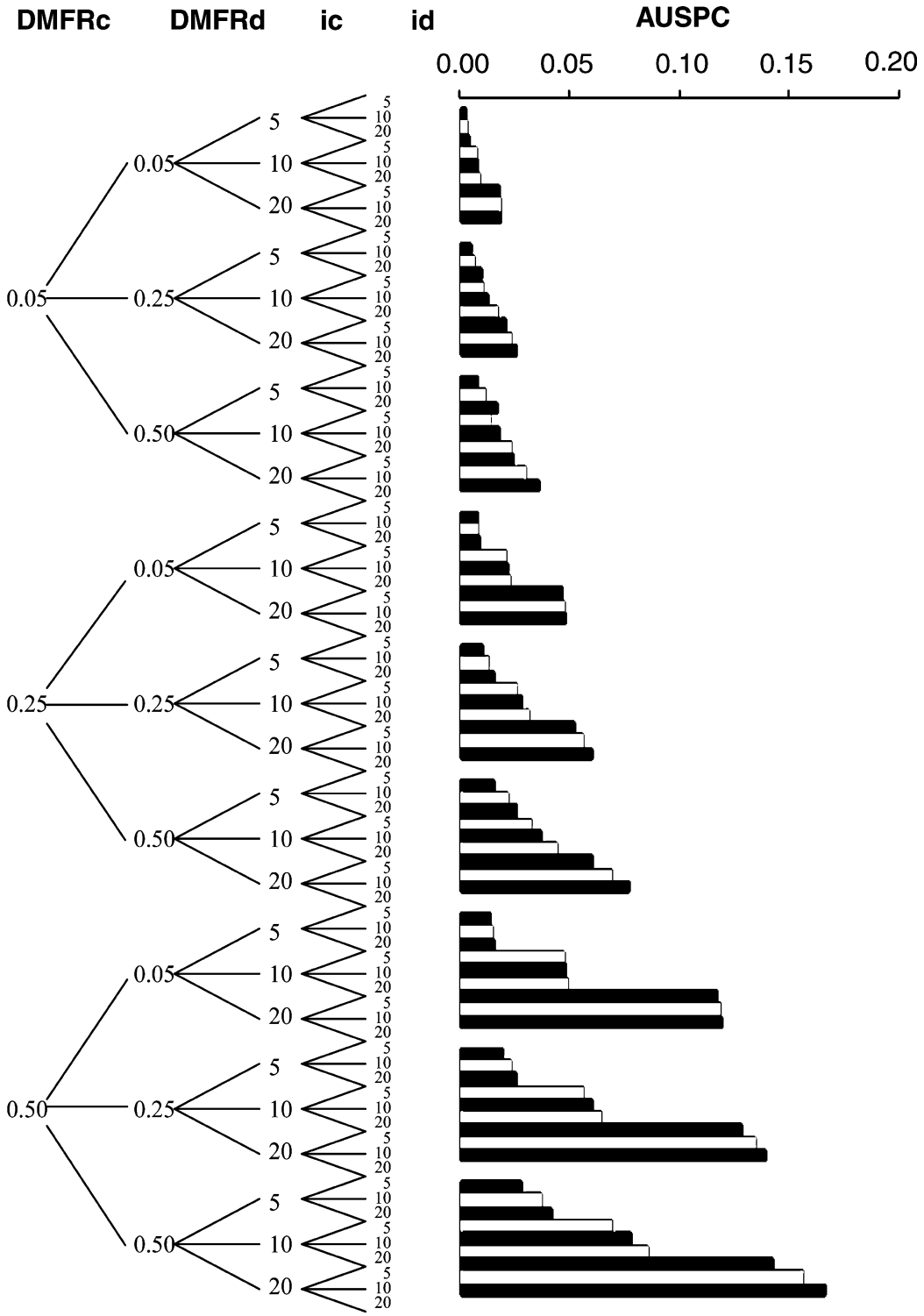

Figure 5. Simulated area under severity progress curves (AUSPC) according to the combination of the daily multiplication factors and the infectious periods. DMFRc and DMFRd, daily multiplication factors of infectious sites of canopy leaves and defoliated leaves respectively, ic and id, infectious periods of infectious sites of canopy leaves and defoliated leaves respectively.

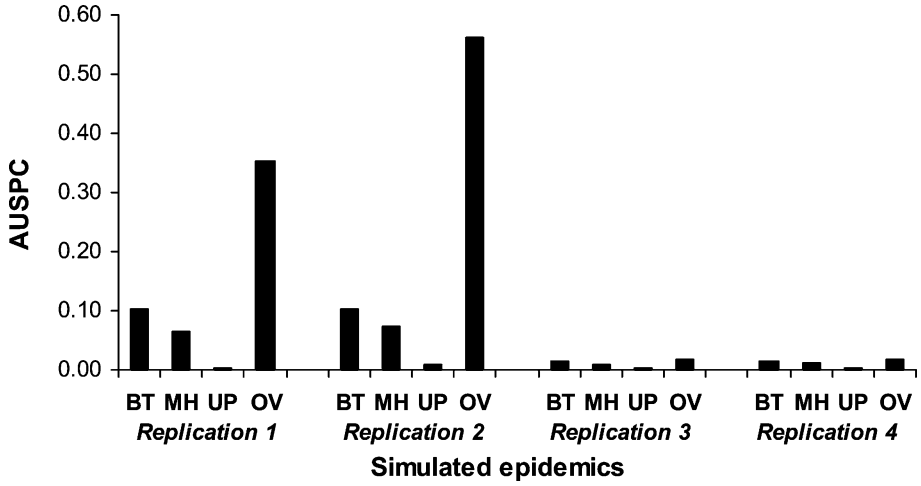

Figure 6. Area under severity progress curves (AUSPC) according to the simulated epidemics. BT, bottom, MH, mid-height, and UP, upper leaf layers; OV, overall, refers to estimates on the entire canopy (leaf layers BT, MH, and UP); see Table 4 for experimental values of daily multiplication factors, DMFRc and DMFRd.

the disease. AUSPC values were also the largest where DMFRc and DMFRd measurements were the largest, i.e. in the two first replications. Even when measured values for DMFRc and DMFRd were zero (UP layer in replication 4), AUSPC was not zero, as simulations were initiated with an inflow of latent lesions at $t = 30$ days after sowing.

Discussion

This work provides quantitative experimental estimates of daily multiplication factors (DMFR) involving two sources of inoculum in a pathosystem, and numerical outcomes of these DMFR values in simulated epidemics. Very little, if any, experimental measures of DMFR exist in the literature, in spite of their conceptual importance since the 'classical' theory for botanical epidemiology developed by Van der Plank (1963) and the companion simulation approach developed by Zadoks (1971). One important question this work raises is whether the empirical measures we derived result in viable epidemics, and how these values compare with those that have been used in the literature for the past 40 years.

In this work, our approach was to conduct a simple monocyclic experiment with replications under varying environmental conditions with the aim of measuring a range of DMFRc and DMFRd values; and to assess the polycyclic consequences of these measurements in simulated epidemics using a mechanistic model. One important point is that our experimental approach addresses only one of the two components of DMFR, since: $\mathrm{DMFR} = \mathrm{Rc} = N^{*}E$ (Van der Plank, 1963; Zadoks, 1971; Zadoks and Schein, 1979), where N is the amount of (effective) propagules produced per mother lesion per unit time (dimension: $[N_{\mathrm{spore}}\ {N_{\mathrm{lesion}}}^{-1}\ \mathrm{T}^{-1}]$), and E is infection efficiency ($[N_{\mathrm{lesion}}\ {N_{\mathrm{spore}}}^{-1}]$). Our experiments did not address variation in E, as incubation was standardised under semi-controlled conditions, and therefore was identical for all leaf layers and treatments. The experiment reported here focused on the other component, N, which not only represents the amount of propagules produced per lesion per time unit, but also the efficiency by which propagules are transferred from mother lesions to new sites where they possibly can infect if the site is vacant (i.e., has not been infected already), with efficiency E. Infection efficiency, especially for aerial pathogens, is a well-defined concept, that has operational definitions implemented with relative ease for a long time. In the case of ALS of bean, E ranges from 0 to 0.5 (Willocquet et al., 2004). E can only vary between 0 and 1, whereas N has an open upper range of variation. We thus focused on N, a very important

factor in the particular case of ALS, but also a central monocyclic parameter to analyse and understand DMFR effects on disease epidemics.

Sun and Zeng (1994) derived a method to estimate DMFR values. Their method strongly differed from the approach described in the present work in two main ways. First, the approach used by Sun and Zeng (1994) was grounded on Van der Plank's equations (Van der Plank, 1963, 1965), and therefore addressed an entire epidemic; here we quantified the outcome of one wave of dispersing propagules from a quantified inoculum source for a substantial, but limited, period of time (4 days). Second, the work by Sun and Zeng (1994) was based on the assumption of a single source of inoculum ensuring disease progress, whereas the present work considered two different sources in a diseased canopy. Interestingly, Sun and Zeng's estimates of DMFR range from 1 to 80, an extremely high value, which suggests that an analytical approach to address this question, and which encompasses an entire epidemic, might generate parameters that reflect numerical trends only. The maximum DMFR values experimentally measured in this work are approximately 1 (DMFRc) and 0.5 lesion lesion^{-1} day^{-1} (DMFRd). These values are smaller than those that have commonly been used in mechanistic simulation models, e.g. 8 and 16 (Zadoks, 1971; in a generic mechanistic epidemiological model), 10 (Mundt et al., 1986; Mundt and Brophy, 1988, in a mechanistic model for wheat stem rust and oat crown rust), 2–35 (Lannou et al., 1994, in a mechanistic model for wheat yellow rust). DMFR values have also been indirectly estimated from simulated outputs themselves by Savary et al. (1990): 0–6 under dry and 1–72 under rainy conditions for groundnut rust. The values generated from the experiments reported in the present work therefore appeared small compared to those commonly used. While Figure 4 suggests that such values translate into terminal (maximum) severities that do not exceed 2% (and a maximum severity of about 11% using experimentally measured parameters; simulation outputs not shown) these DMFR values may however correspond to very large defoliation fractions (of up to 30%, Figure 4) which are well in the range of observed epidemics.

The significant inoculum treatment effect on daughter lesion density (Figure 2) can be explained in two ways: first, the amount of mother lesions in T3 (canopy and ground) was generally higher than that in treatments T1 (canopy) or T2 (ground); and second, mother lesions in T1 appeared to be more efficient in generating daughter lesions than T2. The significant leaf layer effect derived from a higher density of daughter lesions which generally appeared in the upper (Figure 2, UP) and middle (Figure 2, MH) layer of the canopy, compared to the bottom layer (BT).

Our results suggested that comparisons between DMFRc and DMFRd indicated canopy-borne inoculum contributed to more new lesions than inoculum generated on defoliated infectious tissues. Several reasons may explain the difference, including (i) lesser mobilisation of inoculum on the ground (at least under dry conditions), (ii) lesser accessibility of healthy sites to propagules emitted from lesions on the ground compared to propagules produced by lesions in the canopy, (iii) a buffering effect of the lower layers of the canopy to propagules that are emitted by the ground source. Large differences in DMFR values were found between replications 1 and 2, and replications 3 and 4. This may be attributed to the occurrence of rainfall events during the first two replications, which (i) contributed to canopy-borne inoculum (Savary et al., 2004) and (ii) enabled dispersal of spores from lesions on the ground. In the latter case, rainfall may disperse spores up to 40 cm vertically within the canopy. Dry conditions (replications 3 and 4) did not appear to favour dry spore dispersal within the canopy (low DMFRc values), and prevented spore dispersal from lesions on the ground (DMFRd values equal or close to 0). Our results showed that inoculum generated on defoliated infectious tissues, as well as canopy-borne inoculum, contributed to new infections in the canopy especially during rainfall events.

The progeny per parent ratio (Van der Plank, 1963, Zadoks and Schein, 1979) can be calculated in the case of this model with a dual source of inoculum as:

$$(\mathrm{DMFRc} \cdot \mathrm{ic}) + (\mathrm{DMFRd} \cdot \mathrm{id})$$

Assuming infectious periods (ic and id) of 20 days, the threshold beyond which an epidemic may take place, i.e. when the progeny per parent ratio exceeds unity, was not passed in replication 3 (in any layer or for the overall canopy), nor for layers BT

and UP of replication 4. This agreed with simulation results at the polycyclic scale (Figure 6), where areas under severity progress curves remained low for simulations using parameters derived from experiments 3 and 4. The threshold theory has been largely explored in human and animal epidemiology (e.g. Anderson and May, 1979), and synthesised as $R_0 > 1$ where R_0 is the average number of new infections produced when an infectious individual is introduced into a susceptible population. R_0 is the equivalent of Rc.i introduced by Van der Plank (1963) and used later by Zadoks and Schein (1979) and Jeger (1986) to address threshold criteria in plant disease epidemics. Expressions for threshold criteria have recently been proposed in the case of botanical epidemics with dual sources of infection (primary and secondary; Gubbins et al., 2000; Gilligan, 2002). One conclusion of this latter work was that epidemics can develop even if the pathogen cannot invade from primary or secondary infection alone. A similar conclusion may be derived in the case of ALS, where epidemics can occur even if thresholds are not reached for each source of inoculum separately (lesions on attached and defoliated leaves).

The mechanistic simulation model developed here enables projecting the effects of DMFR values throughout a polycyclic process, and comparing the effects of two inoculum sources, lesions in the canopy, and lesions on defoliated leaves. Among the many hypotheses underlying our simulation model, one is the absence of mortality (defoliation) of latent sites. It is logical to consider that a single leaflet can bear a number of healthy, latent, infectious, and removed sites; all four categories should therefore be exposed to defoliation when it occurs. The model developed here only considers two, coupled, defoliation processes: on the infectious and on the healthy sites. The first lesions to develop on an infected leaflet are latent sites. Omitting a defoliation process for the latent sites suggests that they cannot cause defoliation affecting themselves or neighbouring healthy sites, or conversely, that it takes a latency period for these lesions to cause leaf tissues to be shed. The model therefore implicitly assumes that the presence of infectious lesions is required to cause defoliation, which simultaneously affects neighbouring healthy tissues. This hypothesis also reflects the fact that severity, i.e. the proportion of visibly infected tissues, is the basis for computing defoliation. Another interpretation of the structure we chose for this model is that we intentionally prevent latent sites from death: implicitly, one may assume that a majority of latent lesions are established on tissues that were previously healthy (leaflets), i.e. that latent sites reflect colonisation. This other interpretation considers two main categories of tissues in a diseased canopy: a healthy group of tissues, where only latents may establish, and where no defoliation occurs, and a diseased group of tissues where infectious sites are responsible for defoliation that also affects healthy sites. This kind of stratification corresponds to reality in this type of disease (Plaut and Berger, 1980; Savary et al. 1990), where a vertical disease gradient is commonly observed.

Several elements suggest that the model used here has value in extrapolating monocyclic data to polycyclic outcomes, in spite of its simplicity. First, several runs indicated that the model behaves adequately with respect to severity increase, variation in healthy sites, and evolution of infected sites (latent, infectious-attached, infectious-detached, removed-attached, and removed-detached sites). Second, the simulated terminal severities that were obtained using experimentally-derived parameters (with a maximum of about 11%) were within the range (5–29%) of those reported figures in the literature (Mora et al., 1985; Buruchara et al., 1988; Bergamin-Filho et al., 1997; Carneiro et al., 1997; Silva et al 1998; de Jesus Junior et al., 2001, 2003). Such comparatively low severities can be attributed to the opposite effects of host growth and (disease induced) defoliation. This has been described in other pathosystems, such as clover leaf spot (Nelson and Campbell, 1993), several leaf spot diseases of alfalfa (Thal and Campbell, 1988) or white clover (Nelson and Campbell, 1991, 1993). Severity progress in Figure 4b exhibits fluctuations, as a result of interacting parameters: crop growth, defoliation, (high) DMFRc and DMFRd values and (low) ic and id values. Such fluctuations are likely to occur in a system where the disease influences the amount of healthy sites in a way other than infection itself.

Acknowledgements

This research was partly supported by the European Project INCO contract No. ICA4-CT-2000–30004 between IRD (Institut de Recherche pour le Développement), EMBRAPA Arroz e Feijão, the Phytopathology Unit of Gembloux University, and CATIE (Centro Agronómico Tropical Investigación y Enseñanza).

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