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MODELLING PROPAGULE EFFECTS IN BEAN WHITE MOULD EPIDEMICS

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Tese apresentada à Universidade de Brasília como requisito parcial para a obtenção do título de Doutor em Fitopatologia pelo Programa de Pós Graduação em Fitopatologia

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Aos meus avós maternos Osvaldo Maia (*in memorian*) e Maria Maia e avós paternos Otávio Geraldine (*in memorian*) e Olinda Lopes de Araújo (*in memorian*) dedico este trabalho por sempre lembrarem meus pais Arlinda Maia Geraldine e Alaerson Gomes Geraldine a importância dos estudos.

Dedicatória

Trabalho realizado junto ao Programa de Pós-graduação em Fitopatologia do Instituto de Ciências Biológicas da Universidade de Brasília, sob orientação do **Professor Adalberto Corrêa Café Filho e co-orientação do Dr. Serge Savary** com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Embrapa Arroz e Feijão e Institut National de la Recherche Agronomique (INRA).

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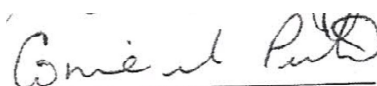
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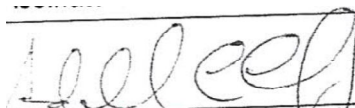
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RESUMO GERAL DA TESE

GERALDINE, Alaerson Maia. **Modelagem do efeito dos propágulos em epidemias de mofo-branco do feijoeiro**. 2015. (160p). Tese (Doutorado em Fitopatologia) – Universidade de Brasília, Brasília, DF.

Apesar do grande número de estudos sobre doenças causadas por *Sclerotinia sclerotiorum*, os processos componentes do ciclo das doenças nos diferentes hospedeiros são quase sempre estudados independentemente. O ciclo das doenças causadas por *Sclerotinia* é bastante complexo, e pode ser dividido em quatro fases, correspondendo a estruturas típicas do ciclo de vida do patógeno. Essas fases correspondem à formação e função biológica de quatro tipos de propágulos: (1) escleródios, (2) apotécios e ascósporos, (3) pétalas infectadas, e (4) hifas infecciosas. Um etográfico serve para sintetizar o conhecimento relevante sobre a epidemiologia de uma doença. No primeiro capítulo, versões sucessivas de um etográfico são usadas para discutir a fenomenologia do mofo-branco do feijoeiro, enfatizando as relações entre propágulos de *S. sclerotiorum*, os sucessivos estágios da doença e os estágios de desenvolvimento do hospedeiro. O etográfico descreve a fenomenologia do mofo-branco do feijoeiro através do exame dos vários estágios do ciclo da doença em maior detalhamento do que geralmente é apresentado, e leva em consideração a concatenação de eventos pertinente ao ciclo. O método é útil em identificar os estágios-chave do ciclo e em explicitar as relações entre os estágios da doença, os quatro tipos de propágulos e os fatores que afetam seus respectivos processos de infecção. A abordagem também é útil como primeiro passo para modelagem quantitativa do mofo-branco do feijoeiro. No capítulo 2, foram analisados os estágios sucessivos (fases das epidemias), bem como seus processos de regulação e fatores envolvidos. Foram estudadas epidemias em lavouras de feijão, em condições ambientais naturais prevalecentes no período de inverno no Brasil Central. Os conjuntos de epidemias experimentais objetivaram promover uma série de epidemias de mofo branco. Cinquenta e sete epidemias, representando uma grande variação em níveis de severidade e formas de progresso da doença foram monitorados com atenção específica para os quatro tipos de propágulos considerados. As epidemias foram analisadas por diferentes técnicas estatísticas e três grupos (A, B e C) de epidemias foram identificados. O Grupo A inclui epidemias de lento estabelecimento, desenvolvidas em uma baixa taxa de infecção e levaram a incidências e severidades finais muito baixas. As epidemias no Grupo C tiveram um início precoce, progrediram muito rapidamente com uma taxa de infecção inicialmente elevada, seguida de estabilização. As epidemias no Grupo B apresentaram um comportamento intermediário, inicialmente com baixa taxa de progresso da doença, seguido por um forte aumento da taxa de infecção da folhagem em fases posteriores. Supõe-se que a infeção planta-a-planta (infecção secundária) ocorre raramente em epidemias do grupo A devido menor frequência de contatos efetivos, resultando em um pequeno número de lesões na folhagem e baixa incidência de plantas doentes (média de 12% de incidência). Por outro lado, presume-se que, em epidemias do Grupo C, infecções planta-a-planta ocorrem com frequência, devido maior frequência de contatos efetivos, que conduz a um número muito maior de lesões e maior incidência de plantas doentes (média de 81% de plantas doentes). Portanto, mesmo considerando a relevância do inóculo primário na forma de escleródios e a subsequente formação de apotécios, o papel das infecções subsequentes por outros propágulos em epidemias de mofo-branco deve ser levado em conta. No capítulo 3 é descrita a estrutura de um modelo de epidemias de mofo-branco em feijão que enfatiza os

sucessivos tipos de propágulos do patógeno e sua interação com plantas hospedeiras. Um etográfico descrevendo o ciclo de infecção em feijão mofo-branco foi desenvolvido utilizando símbolos e conceitos desenvolvidos para análise de sistemas. Em seguida um modelo simplificado foi construído utilizando o programa STELLA® 10,6 (Isee system, EUA). Em seguida, oito parâmetros do modelo foram ajustados para variar em quatro níveis. Análises de sensibilidade e métodos estatísticos foram então conduzidos a fim de identificar lacunas, quais parâmetros mais contribuem para variabilidade dos resultados e quais parâmetros são altamente correlacionados com as saídas do modelo. A análise de sensibilidade do sub-modelo de germinação de escleródios mostrou que mudanças na taxa relativa de escleródios germinados (RRGSC) causou variação na área abaixo da curva de progresso da doença (AUDPC), que foram menores do que os causados pela variação no número de inicial de escleródios (SC). A análise de sensibilidade do sub-modelo de infecção de flor mostrou que alterações nos valores da taxa relativa de senescência floral (RRFS) causou menor variação na AUDPC do que mudanças na taxa relativa de infecção floral (RRFI). No terceiro sub-modelo de simulação de infecção da folhagem de feijão, a taxa relativa de infecção primária na folhagem (RRP) teve o mais forte efeito sobre os valores AUDPC. Diferentes análises (análise de variância e análise de componentes principais) indicam que SC, RRGS, RRFI, RRP e RRS foram os parâmetros mais importantes do modelo de simulação de epidemias de mofo-branco. Três grupos de epidemias foram estabelecidas utilizando os resultados do modelo (valores de AUDPC) como um critério para a formação de grupos. Como resultados, os três grupos de epidemias foram confirmados pela análise de discriminante envolvendo uma combinação de parâmetros do modelo de simulação mofo branco. Claramente, o patossistema *Phaseolus vulgaris - Sclerotinia sclerotiorum* é muito mais complexo do que o modelo teórico geral para doenças "de juros simples", mesmo que a quantidade de inóculo inicial (na forma de propágulo 1, escleródios) permaneça como um dos mais importantes parâmetros, como mostrado por análise de sensibilidade.

Palavras-chave: disseminação planta-a-planta, hifas infecciosas, infecção de flores, *Phaseolus vulgaris*, propágulos, *Sclerotinia*.

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THESIS GENERAL ABSTRACT

GERALDINE, Alaerson Maia. **Modelling propagule effects in bean white mould epidemics**. 2015. (160p). Thesis (Doctorate in Plant Pathology) – Universidade de Brasilia, Brasilia, DF, Brazil.

Despite the large number of studies on *Sclerotinia* diseases on several hosts, the processes composing disease cycles are almost always studied separately. The cycle of *Sclerotinia* diseases is complex, and can be divided in four broad phases, corresponding to typical structures of the pathogen life cycle. These phases correspond to the formation and the biological functions of four different types of propagules: (1) sclerotia, (2) apothecia and ascospores, (3) infected petals, and (4) infectious hyphae. An ethograph graphically synthesizes knowledge relevant to epidemiology of a disease. In the first chapter, successive versions of an ethograph are used to discuss the phenomenology of the bean white mould cycle and to highlight relationships among *S. sclerotiorum* propagules, successive stages of the disease and bean developmental stages. The ethograph describe the phenomenology of bean white mould through the examination of the several stages of the disease cycle in more detail than it is usually attempted and taking into account the concatenation of events. The method is useful to identify key stages and to explicit the relationships among disease stages of the disease cycle, the four types of propagules, and the factors that affect their respective processes of infection. It constitutes an initial step to for the development of a quantitative, simulation model. In Chapter 2 we analysed these successive stage (phases), as well as their regulating processes and factors. Sets of experimental epidemics were created in bean fields under natural environmental conditions prevailing in the winter season in Central Brazil. The sets of experimental epidemics aimed to promote a range of white mould epidemics. Fifty-seven epidemics, representing a wide variation in severity levels and in disease progress shapes, were monitored, with specific attention paid to the four considered propagule types. Epidemics were analysed by different statistical techniques and three groups (A, B, or C) of epidemics were distinguished. Group A includes epidemics which were slow to establish, developed at a low rate, and led to very low terminal incidence and severity. Epidemics in Group C had an earlier onset, progressed very rapidly with an initially high rate of increase, followed by stabilization. Epidemics in Group B displayed an intermediate behaviour, with an initially low rate of disease increase, followed by a strong increase of the rate of foliage infection at later stages. It is assumed that plant-to-plant spread (secondary infection) occurs rarely in Group A epidemics because of the lower frequency of effective contacts, resulting into a small number of lesions on the foliage and low incidence of diseased plants (average 0.12% of incidence). By contrast, it is assumed that, in epidemics of Group C, plant-to-plant spread occurs frequently, because of the higher frequency of effective contacts, leading to a much higher number of lesions and higher incidence of diseased plants (average 0.81% of incidence). Therefore, even if the primary inoculum in the form of sclerotia and the subsequent formation of apothecia are, in part, relevant, the role of subsequent infections by other propagules on white mould epidemics must be taken into account. In chapter 3 we described the structure of a model for bean white mould epidemics that emphasizes on the successive types of pathogen propagules and their interaction with host plants. An ethograph describing the infection cycle in bean white mould was developed using symbols and concepts developed for system analysis and a simplified model was built using the STELLA® 10.6 programme (Isee systems, USA). Next, eight model parameters were set to vary in four levels for model evaluation. Sensitivity

analyses and statistical methods were then conducted in order to identify gaps, which inputs contribute most to output variability and which parameters are most highly correlated with the outputs. Sensitivity analysis of the sclerotia germination sub-model showed that changes in the relative rate of germinated sclerotia (RRGSC) caused variation in the area under disease progress curve (AUDPC) that were smaller than those caused by variation in the parameter initial number of sclerotia (SC). Sensitivity analysis of the flower infection sub-model showed that changes in values of the relative rate of flower senescence (RRFS) caused narrower AUDPC variation than changes in the relative rate of flower infection, RRFI. In the third sub-model simulating infection of bean foliage, the relative rate of primary infection on foliage, RRP, had the strongest effect on AUDPC values. Different analyses (analyses of variance and principal component analysis) indicate that SC, RRGS, RRFI, RRP and RRS were the most important parameters of the white mould epidemic simulation model. Three epidemics groups were established using the outputs of the model (AUDPC values) as a criterion for group formation. As a result three epidemic groups were defined by discriminant analysis involving a combination of parameters of the white mould simulation model. Clearly, the bean (*Phaseolus vulgaris*) - *Sclerotinia sclerotiorum* pathosystem is much more complex than the theoretical general "simple interest" diseases model, even if the amount of initial inoculum (in the form of propagule 1, sclerotia) remains as one of the most important parameters, as shown by sensitivity analysis.

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CHAPTER 1

DEVELOPING AN ETHOGRAPH FOR THE BEAN WHITE MOULD PATHOSYSTEM

DEVELOPING AN ETHOGRAPH FOR THE BEAN WHITE MOULD PATHOSYSTEM

ABSTRACT

A very large number of studies have addressed the characteristics of *Sclerotinia sclerotiorum* (Lib.) de Bary and of the diseases it causes on several hosts, such as bean, soybean, lettuce, carrot, sunflower and many other crops. However, the processes composing the disease cycle have been studied separately in these crops. The disease cycle of *Sclerotinia* diseases is complex. This cycle can be divided in four broad phases corresponding to typical pathogen structures of the pathogen life cycle. These phases correspond to the formation and biological functions of four different types of propagules and four stages in the disease cycle are therefore distinguished: (1) sclerotia, (2) ascospores or apothecia, (3) infectious mycelium in the petals, and (4) infectious mycelium in the canopy. An ethograph graphically synthesizes knowledge relevant to epidemiology of a disease. In this study, successive versions of an ethograph were used to discuss the phenomenology of the bean white mould cycle and to highlight relationships among *S. sclerotiorum* propagules, successive stages of the disease, and bean development stages. **The first stage** takes into account the amount of overwintering sclerotia. **The second stage** is characterized by the number of apothecia and ascospores produced at the blooming period. **The third stage** describes the processes involving the number of infected petals at the blooming period. **The fourth stage** concerns the spread of infectious mycelium, which accounts for disease spread from diseased to healthy vegetative tissues. The ethographs developed in this Chapter describe the phenomenology of bean white mould through the examination of the several stages of the disease cycle in more detail than is usually attempted and taking into account the concatenation of events. The building process was especially useful to identify key stages

and to explicit the relationships among disease stages of the disease cycle, the four types of propagules and the factors that affect their respective processes of infection. This approach constitutes an initial step to synthesize epidemiological knowledge of the disease in view of the development of a quantitative simulation model.

Keywords: apothecia, ascospores, infectious hyphae, *Phaseolus* bean, sclerotia.

1. INTRODUCTION

Plant diseases caused by a biotic agent require the interaction of a susceptible host, a virulent pathogen, and a favourable environment for development (Agrios 2005). The phenomenon has long been represented in its simplest qualitative sense by the disease triangle (Stevens 1960). The triangle has been expanded to the tetrahedron allowing for man-made influences such as management practices (Zadocks and Schein 1979). In a quantitative sense, the disease cone (Browning et al. 1977) and the disease prism (Francl 2001) allow for the representation of change in each element over time and quantitative disease expression (Francl 2001). Epidemiologically, plant diseases are usually conceptualized in terms of the key stages, sub-stages and influencing factor where the "infection chain" (Gäumann 1950) represents the basis for understanding the underlying mechanics of plant disease epidemics. The concept of ethographs derives from ethology, as graphical representations of behaviours that are relevant to a particular question that is being asked. Putter (1986, Sixth Epidemiology Workshop, Bet Dagan, pers. comm.) and Savary (2007) called an ethograph a diagram depicting the successive stages of a plant infectious disease, directly linked to epidemic processes and pathogen's reproductive behaviour.

In this review, we make use of series of ethographs of increasing complexity to illustrate and revise knowledge of the key stages and influencing factors of the reproductive and dispersal behavior of the bean white mold pathogen, an ascomycete fungus commonly acknowledged as leading to a monocyclic epidemics. Many reports have described various characteristics of the fungus and of the diseases it causes on bean, soybean (e.g. Teles et al. 2013), lettuce (e.g. Young et al. 2004), carrot (e.g. Parker et al. 2014), sunflower (e.g. Ekins et al. 2011) and several others. For each of these crops, processes composing the disease cycle

have been studied separately, including the processes of sclerotia germination (e.g. Nepal and del Río Mendoza 2012), apothecia formation and ascospore release (e.g. Mila and Yang 2007, Qandah and Del Rio Mendoza 2012). Differently from these previous reviews of the disease cycle, that focused on specific processes, we aimed to highlight and map the components of the multiple interactions (environmental as well as man-made) and their influences on the pathogen, crop and ecosystem in order to improve the understanding of white mold epidemics in agricultural crops.

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal pathogen causing diseases in a wide range of crops. The taxon was first studied by Libert, as *Peziza sclerotiorum* (Libert, 1837, *apud* Bolton et al., 2006). Since then, many reports have described various characteristics of the fungus and of the diseases it causes on several hosts, such as bean, soybean (Teles et al., 2013), lettuce (Young et al., 2004), carrot (Parker et al., 2014), sunflower (Ekins et al., 2011) and many others. For each of these crops, processes composing the disease cycle have been studied separately, including the processes of sclerotia germination (Nepal & del Río Mendoza, 2012), apothecia formation and ascospore release (Mila & Yang, 2007, Qandah & Del Rio Mendoza, 2012).

1.2 Pathogen taxonomy and biology

The pathogen belongs in the phylum *Ascomycota*, class *Discomycetes*, order *Helotiales*, family *Sclerotiniaceae* and genus *Sclerotinia* (Bolton et al., 2006). Three species (*S. sclerotiorum*, *S. trifoliorum* and *S. minor*) have been proposed in *Sclerotinia*, although Purdy (1955) included them all in *S. sclerotiorum.* Subsequently, Willettsh and Wong (1971) and Wong and Willettsh (1973) found sufficient grounds to reinstate the three distinct taxa, which are valid today (Kirk et al., 2008). *Sclerotinia sclerotiorum* [*nom. cons.*, syn. *Whetzelinia sclerotiorun* (Lib.) (Korf & Dumont, 1972)] is characterized by dikaryotic

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ascospore cells and a haploid chromosome number of eight (Wong & Willetts, 1979). By contrast, the other two species have four nuclei per ascospore. Additional cytological differences supported the distinction of *S. sclerotiorum*, *S. minor* and S. *trifoliorum* as different species (Wong & Willetts, 1979). The sexual cycle of *S. sclerotiorum* is characterized by the formation of apothecia, which germinate carpogenically from sclerotia and form sexual spores (ascospores) in asci (Figure 1.B1). Each ascus contains eight spores that are produced by meiosis followed by a mitotic division (Figure 1.B2). The asci eventually forcibly puff-off the ascospores into the air (Figure 1.B2), and up to 2 x 10^6 ascospores are formed per apothecium (Schwartz & Steadman, 1978). *S. sclerotiorum* is also characterized by hyaline, septate, branched and multinucleate hyphae (Bolton et al., 2006) and hyphal masses that mature into hard black sclerotia that can survive long periods of time in the soil (Figure 1.A). Contrary to many plant pathogenic fungi, no asexual conidia are produced by *S. sclerotiorum* (Bolton et al., 2006).

1.3 Host plants and damage caused by white mould epidemics

Sclerotinia sclerotiorum causes diseases on more than 400 botanical species (Boland & Hall, 1994) and is an important pathogen of many agricultural crops in Brazil and worldwide. The economic importance of *S. sclerotiorum* is well documented in Boland & Hall (1994). According to these authors, plant families that contain the largest numbers of hosts include *Fabaceae*, *Brassicaceae* and *Solanaceae*. *Fabaceae* includes at least 61 hosts of *S. sclerotiorum* (Boland & Hall, 1994), with many economically important edible and industrial oil and grain crops, such as lentil (*Lathyrus esculenta* Moench.), cowpea (Vigna *unguiculata* (L.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), peanut (*Arachis hypogaea* L.*)* and soybean (*Glycine max* (L.) Merrill). *Brassicaceae* contains 48 hosts (Boland & Hall, 1994), with many key edible and industrial oilseed, vegetable and fodder crops, such as canola (*Brassica*

napus L.), kale, cabbage, cauliflower, and broccoli (several varieties of *Brassica oleracea* L.), among others. *Solanaceae* hosts comprise many significant vegetable crops, including potato (*Solanum tuberosum* L.), tomato (*S. lycopersicon (*Mill.)), tobacco (*Nicotiana tabacum* L.), eggplant (*S. melongena*), and hot and sweet pepper (*Capsicum annuum* L.) (Boland & Hall, 1994). Among other economically important hosts, the Asteraceae sunflower (*Helianthus annuus* L.), and lettuce (*Lactucae sativa* L.) are worth mentioning (Heffer Link & Johnson, 2007).

Phaseolus vulgaris L. (common bean) is a leguminous crop which is a key food crop in Latin America and the tropical world (*e.g.* East Africa). Brazil is currently the world's third largest edible bean producer and consumer, with a domestic *Phaseolus* production of 2.89 million tons in 2013 (FAO, 2015). In 2015 bean production is expected to reach 3.32 million tons over a planted area estimated at 3.18 million ha in 2014/15 (CONAB, 2015).

Phaseolus bean is grown practically all over the Brazilian territory, in three distinctive seasons. For the first (rainy-season crop), beans are sown between August and October, and harvested is from November through February. In the second season (dry-season crop) bean sowing takes place from December to March and harvest is from March through June. For the third season (winter crop), bean is sown from May through July, is irrigated either by pivot or sprinkler systems (Oliveira et al., 2013), and is harvest from August to September.

The irrigated winter bean crop (28.7 % of the annual production in 2014 (CONAB, 2015)) is characterized by dry weather and low temperatures, and is known to be the bean crop most affected by white mould in Brazil. White mould epidemics are usually associated to increased soil moisture and mild temperatures, typical of the winter season. High canopy wetness and soil moisture are brought by irrigation. Soil moisture is important for carpogenic germination of sclerotia, with water potentials more humid than -1.5 MPa

generally required for the germination process to start (Hao et al., 2003). The disease causes substantial annual losses of common beans in all major producing regions of the world (Boland & Hall, 1987a, Boland & Hall, 1987b, Ramasubramaniam et al., 2008) which sometimes reach up to 100% of the yield (Lobo Junior et al., 2009).

1.4 Disease cycle

A number of distinct pathogen structures are involved in the life cycle of *S. sclerotiorum* on beans: sclerotia, apothecia, ascospores and mycelium (Figure 2A; Figure 3). The same pathogen structures are involved in the life cycle of *S. sclerotiorum* on many different host plants. For example, the life cycle of white mould on potato and soybean are similar (Figure 2). Firstly, sclerotia are the primary inoculum, since they play a major role as survival structures (Willetts and Wong, 1980) between cropping seasons, remaining viable for up to 8 years in soil (Figure 1.A) (Adams and Ayers, 1979). Sclerotia germinate either carpogenically (Figure 1.B1) or myceliogenically (Figure 3) (Purdy, 1979). Myceliogenic germination of sclerotia leads to the production of hyphae that can infect plant tissues directly (Bardin and Huang, 2001). This type of germination appears to be of minor importance in bean crops. For example, Tu (1989) found that infections may occur when leaves come into contact with sclerotia on the soil surface, but did not consider this type of infection of epidemic significance. Generally, diseases caused by *S. sclerotiorum* are initiated by ascospores coming in contact with and colonizing senescent plant tissue such as flowers (Figure 1.C)beneath the host plant canopy (Schwartz & Steadman, 1978, Abawi & Grogan, 1979a). White mould epidemics generally start when sclerotia germinate carpogenically, producing apothecia. After apothecium and ascus formation (Figure 1.B1 and B2), subsequent subprocesses include ascospore ejection into the air, dispersal and deposition.

In beans and many other crops, ascospores are not thought to infect plant foliage directly, but via infected petals which act as nutrient sources (Abawi & Grogan, 1979a). When ascospore-infected petals are shed and deposited on leaves, *S. sclerotiorum* invades the vegetative tissues, causing disease lesions on foliage (Schwartz & Steadman, 1978, Clarkson et al., 2014). Phillips (1987) and others, have shown that white mould infections are mostly due to carpogenic germination of sclerotia and subsequent infection of petals, with senescent flower tissues serving as nutrient sources for ascospore germination and infection. The location of initial infections in axillary tissues at the site of lodgement of cast petals indicates that these petals are essential for the infection processes in vegetative tissues (Natti, 1971).

As sheath blight epidemics on rice, caused by *Rhizoctonia solani (*telomorph*: Thanatephorus cucumeris)* (Savary et al., 1997) and web blight on *Phaseolus* beans, also caused by *T. cucumeris* (Costa-Coelho et al., 2014), secondary infections can develop by spread of mycelium from diseased to healthy tissues. Savary et al. (1997) coined the term "canopy-borne diseases" to refer to this mechanism. Many authors have considered the plant-to-plant spread of *S. sclerotiorum* as a relatively minor portion of the epidemiology of disease (Boland & Hall, 1987a, Tu, 1989, Heffer Link & Johnson, 2007). To others Abawi and Grogan (1979a), however, secondary spread by plant to plant mycelial growth more important on dry beans as a result of the longer period of susceptibility due to indeterminate flowering and the 'moist-chamber effect' produced by the dense canopy of *Phaseolus* foliage.

Following vegetative tissue infection, white cottony mycelia are formed, which will ultimately mature into hard black sclerotia which can initiate in the next crop cycle.

2. DISTINGUISHING FOUR STAGES IN THE WHITE MOULD CYCLE

The life cycle of *S. sclerotiorum* is complex, and can be divided in four broad phases. These phases correspond to the formation and the biological functions of four different types of propagules: sclerotia, ascospores (produced in apothecia), infectious mycelium in petal, and infectious mycelium in vegetative tissue, which grow and spread in the host canopy. These stages correspond to propagules that differ morphologically, genetically, in ontogeny, and in terms biological function to such a degree that their distinction may lead to a better understanding of the phenomenology of bean white mould.

Four stages in the disease cycle are therefore distinguished by their respective propagules: (1) sclerotia, (2) ascospores (from apothecia), (3) infectious mycelium in petal, and (4) infectious mycelium in the canopy (Figure 4).

These stages have been studied separately to varying degrees by numerous authors, but an attempt to bring all successive, concatenated stages of the disease cycle in the same framework is needed. The synthesis below is intended to bring the information on these different stages of the pathogen life cycle to a similar level of precision, in terms of the processes involved and the factors that may affect them. The development of ethographs is used to achieve this purpose.

3. PHENOMENOLOGY AND ETHOGRAPH AND OF WHITE MOULD DISEASE BY STAGES

An ethograph is a tool that graphically summarizes the knowledge relevant to the infection cycle of diseases (Savary, 1987, Savary, 2007). In this study, the building of ethographs was used as a method to discuss the phenomenology of the bean white mould cycle and to demonstrate the relationships among *S. sclerotiorum* propagules, white mould stages and bean development stages (Figures 4; 5; 6; and 7). The first ethograph shows an overall structure of four stages of white mould cycle (Figure 4). **The first stage** represents the amount of overwintering sclerotia. **The second stage** characterizes the number of apothecia

and ascospores produced at the blooming period. **The third stage** symbolizes the number of infected petals at the blooming period, and **the fourth stage** concerns the spread of infectious mycelium, which accounts for disease spread from diseased to healthy vegetative tissues. The second ethograph (Figure 5B) has a structure similar to the overall ethograph, but considers host factors by means of a host time line that represents three important bean developmental stages on disease cycle (seed, full bloom and harvest). These bean developmental stages are illustrated in the ethograph depicted in Figure 7. The ethograph illustrated on Figure 5A emphasizes the relationship between stages of the disease cycle and stages of the bean development together with the relationship between amount of propagules and host development stages. Here, we highlight that the number of infected petals is dependent on the onset of ascospore release and also on the amount of ascospores. The amount of infected petals is proportional to the size of the blue rectangle. According to this diagram, the earlier the release of ascospores (during the blooming period), the greater the amount of infected petals (Figure 5B). A similar relationship can be hypothesized the disease intensity on the foliage. An increase in the amount of infected petals should lead to a proportional increase in the amount of diseased plant parts. Likewise, the greater the amount of canopy-borne infection (mycelial spread), the greater the amount of diseased plants and plant parts at the end of the bean crop season. These relationships are affected by many factors that influence the different stages of white mould epidemics (Figure 6) and will be discussed in each respective process and sub-processes, as necessary.

In the most detailed version of the ethograph (Figure 7), each stage of the white mould epidemic is represented by propagules. Propagule 1 represents existing overwinter sclerotia and new sclerotia (*i.e.* inoculum for the next season). After an average of 35 days from the bean sowing date, roughly coinciding with the bloom period, propagule 1 germinates and

produces propagule 2. Propagule 2 then infects bean petals (propagule 3). The infected petals by mycelium drop onto the bean foliage and infect healthy tissues. Mycelial spread occurs from diseased to healthy tissues by "effective contacts" and is represented by propagule 4. A plant-to-plant spread has been termed "effective contact", *i.e.*, a contact between host and pathogen that leads to disease (Savary et al., 1997). Here, an effective contact is described by a contact between healthy and disease tissues that leads to new infections (Figure 7). Following, each stage of the ethograph depicted in Figure 7 is described: The shaded area in Fig. 7 represents the population of the successive propagules, i.e., the population size of the pathogen.

The population size of the pathogen corresponding to propagule 1 is assumed to remain stable until bean development stage V4. At that point (onset of propagule 2), the area between these two lines increases up to a maximum around bean stage R5, coinciding with blooming period. In the propagule 3 stage, the area between the propagule line and the host time line increases from full bloom period and is maximal at bean stage R6. Consequently, at the propagule 4, the area between lines of propagules and host increases from full bloom period up to maximum infection of vegetative host tissues. The conditions affecting the processes at each stage of the ethograph is discussed in detail:

3.1 Stage 1 – Sclerotia: The process of **sclerotium formation** is influenced by nutrition and pH (Rai & Agnihotri, 1971). The growth of mycelium for sclerotium formation is favoured in pH 4.0 to 6.0 (Rai & Agnihotri, 1971, Rollins & Dickman, 2001). It is noteworthy that the soil population of sclerotia increases in fields where annual epidemics of white mould have occurred (Abawi & Grogan, 1979a).

The ecology of sclerotia and the factors that affect their survival are discussed in many studies (Natti, 1971, Boland & Hall, 1987b, Yang et al., 1990, Wu et al., 2008). Biotic factors

are among the most important for **sclerotium survival and viability**, according to Purdy (1979). *Aspergillus*, *Penicillium*, *Trichoderma* and *Fusarium*, among other fungal *genera*, can parasitize *S. sclerotiorum* sclerotia (Rai & Saxena, 1975, Santos & Dhingra, 1982, Melo et al., 2006), pH, temperature and soil texture can also influence sclerotium survival (Mitchell & Wheeler, 1990, Alexander & Stewart, 1994). Sclerotia of *S. sclerotiorum* produced *in vitro* remained viable for a period of eight months in Cerrado soils, although sclerotium viability depended on the soil type, and was negatively related to the population of soil microorganisms, especially *Trichoderma* (Ferraz et al., 2011). Mitchell and Wheeler (1990) showed that 43% of sclerotia of *S. sclerotiorum* were degraded in a clay loam compared to 59% in bag shot sand after 23 months. Burial depth also influences the survival of sclerotia (Wu et al., 2008): burial at 4 cm for 35 weeks reduced recovery of viable sclerotia to zero in sandy clay loam, but only by 50% in sandy loam (Merriman, 1976). At 35°C, the viability of sclerotia declined rapidly in moist soil. Fewer than 5% of sclerotia remained viable after an 8-week incubation at moisture levels at or above - 0.3 MPa (*i.e.*, soil at field capacity or more), and the percentage of viable sclerotia decreased to near-zero after 12-weeks of incubation (Wu et al., 2008). Similarly, the combination of high temperature, high soil moisture and reduced oxygen in irrigated fields lower survival rates of *S. sclerotiorum* (Wu et al., 2008).

Germination of *S. sclerotiorum* sclerotia may occur either carpogenically (Figure 1.A) or myceliogenically (Bardin and Huang, 2001). Although mycelium growing from sclerotia has been reported to infect beans (Natti, 1971) and other hosts (*e.g.* tomato, Lobo Jr. et al. 2000), it is important to note that sclerotia placed in direct contact with bean tissues generally fail to infect (Abawi & Grogan, 1979a). Carpogenic germination, leading to apothecia production and release ascospores thus appears to be the key contribution of sclerotia to the life cycle of the pathogen.

3.2 Stage 2 – Apothecia: Apothecia and ascospore formation is governed by many of the environmental factors that affect sclerotial germination such as soil temperature, soil moisture and light (Phillips 1987, Bardin & Huang 2001). Temperature during sclerotial formation is an important factor affecting carpogenic germination of sclerotia of *S. sclerotiorum* (Harada et al. 1974; Huang and Kozub 1991). Bedi (1962) determined that the optimum temperature range for apothecia formation is 15-20°C, and Wu and Subbarao (2008) observed that the germination for *S. sclerotiorum* peaked at 15°C and declined only slightly at 20°C. Comparable results were observed by Matheron and Porchas (2005), where germination in irrigated soil was significantly lower at 32°C than at 26°C. Wu and Subbarao (2008) observed that sclerotia of *S. sclerotiorum* germinate carpogenically at temperatures of 22°C at 25°C. Three or more apothecia are usually produced per sclerotium (Saharan & Mehta, 2008).

Moreover, burial depths, temperature, and soil moisture strongly affect carpogenic germination (Phillips, 1986, Clarkson et al., 2004, Wu & Subbarao, 2008). Maximum depth at which a sclerotium of *S. sclerotiorum* produced apothecia was 4.0 cm (Wu & Subbarao, 2008). Under field conditions, Vieira et al. (2012) observed that white mould was favoured by high crop density, extended periods of rainfall, high humidity, and warm temperatures. In these conditions, sclerotia of *S. sclerotiorum* required about 35 days of high soil moisture in the top soil to germinate carpogenically (Wu & Subbarao, 2008).

Apothecia longevity refers to the time period during which ascospores are released from an apothecium. In two different conditions (saturated air, 90–95% RH) and at 65–75% RH, apothecia released ascospores continuously for 15 days under a 12 h photoperiod (Clarkson et al., 2003). However, during a period of 168 h (7 days), there was a significant

decline in the spore number count between 36 h and 168 h at 65–75% RH, whereas no decline was observed at 90–95% RH (Clarkson et al., 2003).

Ascospore germination and viability: Previous *in vitro* work shows that free water (or high humidity) is required for ascospore germination (Abawi & Grogan, 1975, Abawi & Grogan, 1979a, Tu, 1989). Clarkson et al. (2014) estimated the minimum, maximum and optimum temperatures for ascospore germination of 0.0, 29.9 and 21.7°C, respectively. Ascospore survival is reduced at high temperatures and high relative humidity (Caesar & Pearson, 1983).

3.3 Stage 3. Infected petals: Ascospores require exogenous nutrients for infection, which may be in form of senescent flowers for beans and soybeans (Sutton & Deverall, 1983). Germination occurs within 3 h on oilseed rape petals (Jamaux et al., 1995). Similar results were observed by Young et al. (2004), where ascospores started to germinate on lettuce leaves after 2 – 4 h of continuous leaf wetness at temperatures of 15 to 25 °C. Primary infection by means of leaves coming in contact with sclerotia on the soil surface occurs very infrequently, as mentioned (Tu, 1989). The limited hyphal growth emanating from newly produced sclerotia does not appear to play an important role in secondary infections (Steadman, 1983).

Sclerotinia sclerotiorum infection on beans requires plant surface wetness durations of at least 54 h (at 15–25°C) (Abawi & Grogan, 1979b, Boland & Hall, 1987a, Tu, 1989). Bean plants inoculated with *S. sclerotiorum* ascospore suspensions developed disease at RH > 90% at 20–28°C (Hannusch & Boland, 1996) (Figure 5).

3.4 Stage 4. Mycelial spread: A temperature range of 16–22°C is required for an optimum rate of development of sclerotinia disease on beans (Boland & Hall, 1987a, Phillips, 1994). Lesion initiation and development are near optimum at constant temperatures of 10–25°C

(Abawi & Grogan, 1975, Weiss et al., 1980). Environmental conditions in the irrigated winter bean crop in Brazil are favourable to the disease. In addition, traditional Brazilian bean cultivars are of indeterminate growth, with prostrate habit, which are conducive to severe white mould epidemics (Napoleão et al., 2005). On the other hand, in warmer regions where irrigated beans are grown, higher temperatures limit white mould incidence and development (Abawi & Grogan, 1979a). Direct infection of intact leaves are not thought to be significant, since lesions did not develop on inoculated leaves (ascospores) of plants incubated at 5°C or 30°C (Abawi & Grogan, 1975, Weiss et al., 1980).

After growing on host tissues for several days, the mycelium produces sclerotia externally on affected plant parts and/or internally in stem pith cavities, fruit cavities, or between plant tissues (Purdy, 1979). Sclerotia are shed on the soil surface when dislodged by wind or during harvesting and threshing operations (Figure 7) (Schwartz & Steadman, 1978). New sclerotia remain on the field with the crop debris, and may start a new disease cycle in subsequent crops (Schwartz & Steadman, 1978, Grogan, 1979).

4. CONCLUSION

The ethographs have enabled us to: (i) diagrammatically summarize knowledge of a disease cycle, and (ii) highlight and map the multiple interactions: pathogen – host – environment. By 'environment', one should refer to the ecological as well as man-made influences on the pathogen, crop, and ecosystem, so that the ethograph explores the four components of a tetrahedron (Zadoks and Schein 1979).

The several versions of the ethograph describe the phenomenology and reproductive behaviour of the bean white mould pathogen through the examination of the several stages of the disease cycle in detail. Especially, the graphs provide an outline of the concatenation of the successive inoculum types, and the key events and host factors of epidemiological

relevance. The building processes indicated in the ethographs were especially useful to identify main disease cycle stages and to bring forward relationships among disease stages, propagule types and the factors that affect their respective processes of infection. Bean white mould has usually been treated as one example of the monocyclic ('simple interest', *sensu* Van der Plank 1963) model of diseases. Although the importance of the initial inoculum is considerable in this system, the phenomenology of bean white mould is clearly more complex than is usually acknowledged. Mainly, it involves at least four types of functional propagules, repeated infections and amplification of pathogen biomass (for example, by leaf-borne infectious hyphae) and final disease levels that depend on a sequence of concatenated events. Such knowledge is important to guide the conceptualization and elaboration of mechanistic simulations models of white mould epidemics.

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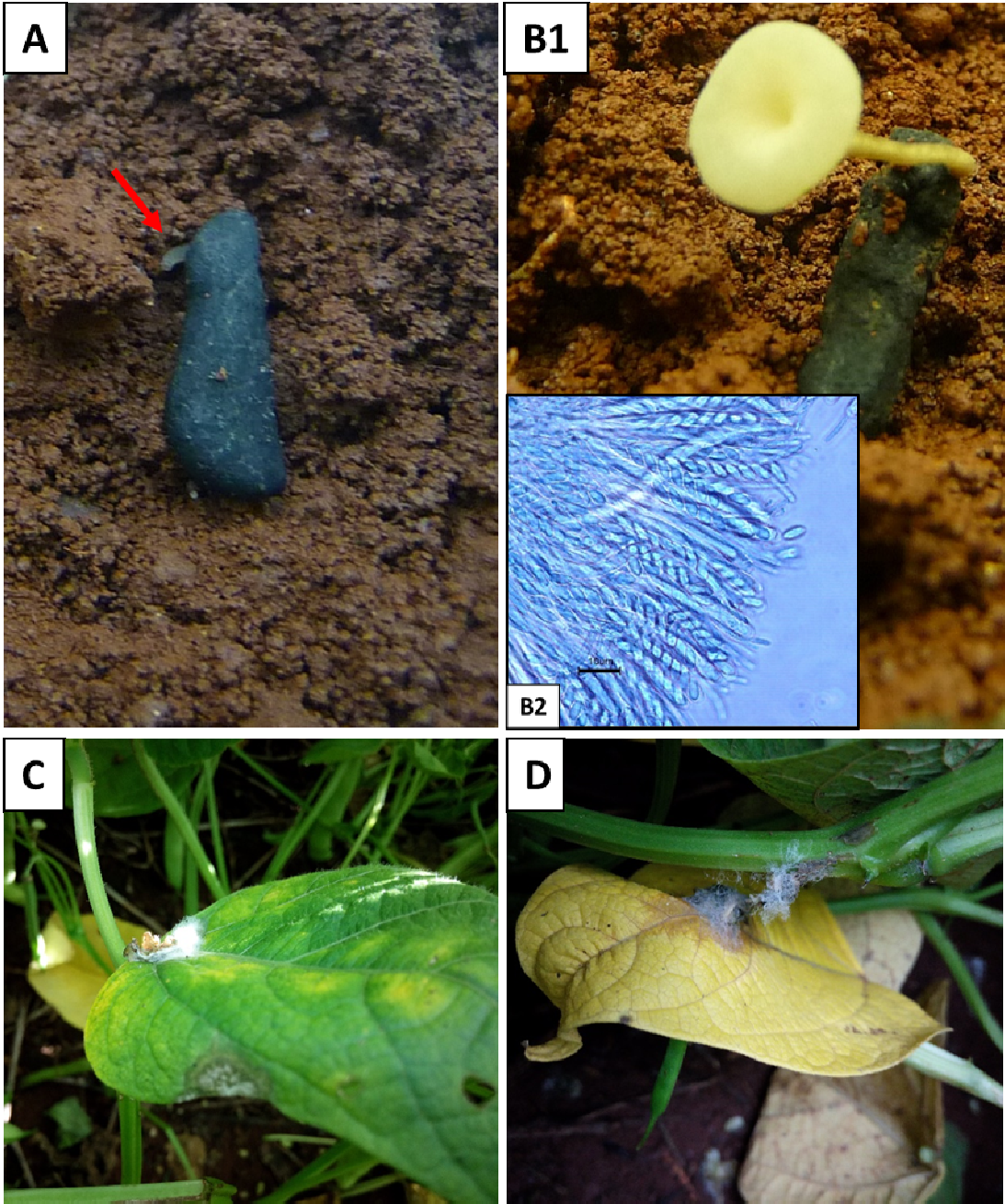
Figure 1. Survival, reproduction and dissemination structures of *S. sclerotiorum:* **A**-Germinating sclerotium (arrow) (Propagule 1); **B1**- Mature apothecium (Propagule 2); **B2**-Asci, paraphyses (Propagule 2) scale 10 μm, **C**- infectious mycelium in petal (Propagule 3) infecting foliage; **D**- infectious mycelium in tissue (Propagule 4) infecting healthy tissue.
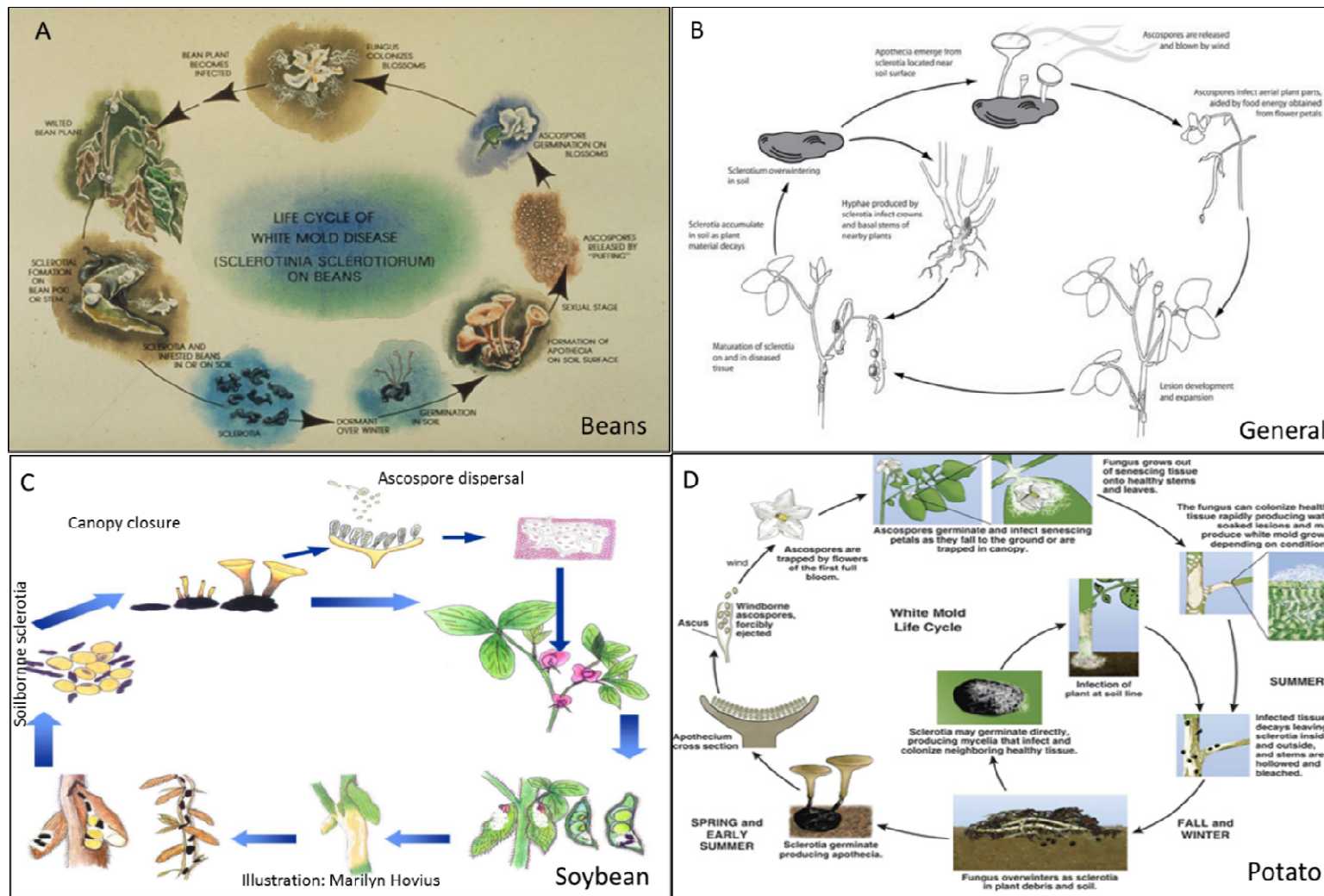
Figure 2. Life cycle of white mould disease (*S. sclerotiorum*) on different host-plants: (A) white mould on beans (Steadman, 1987); (B) Overview cycle (Agrios, 2005); (C) white mould on soybean (PIONNER, 2014) ; (D) white mould on potato (Wharton & Kirk, 2007).
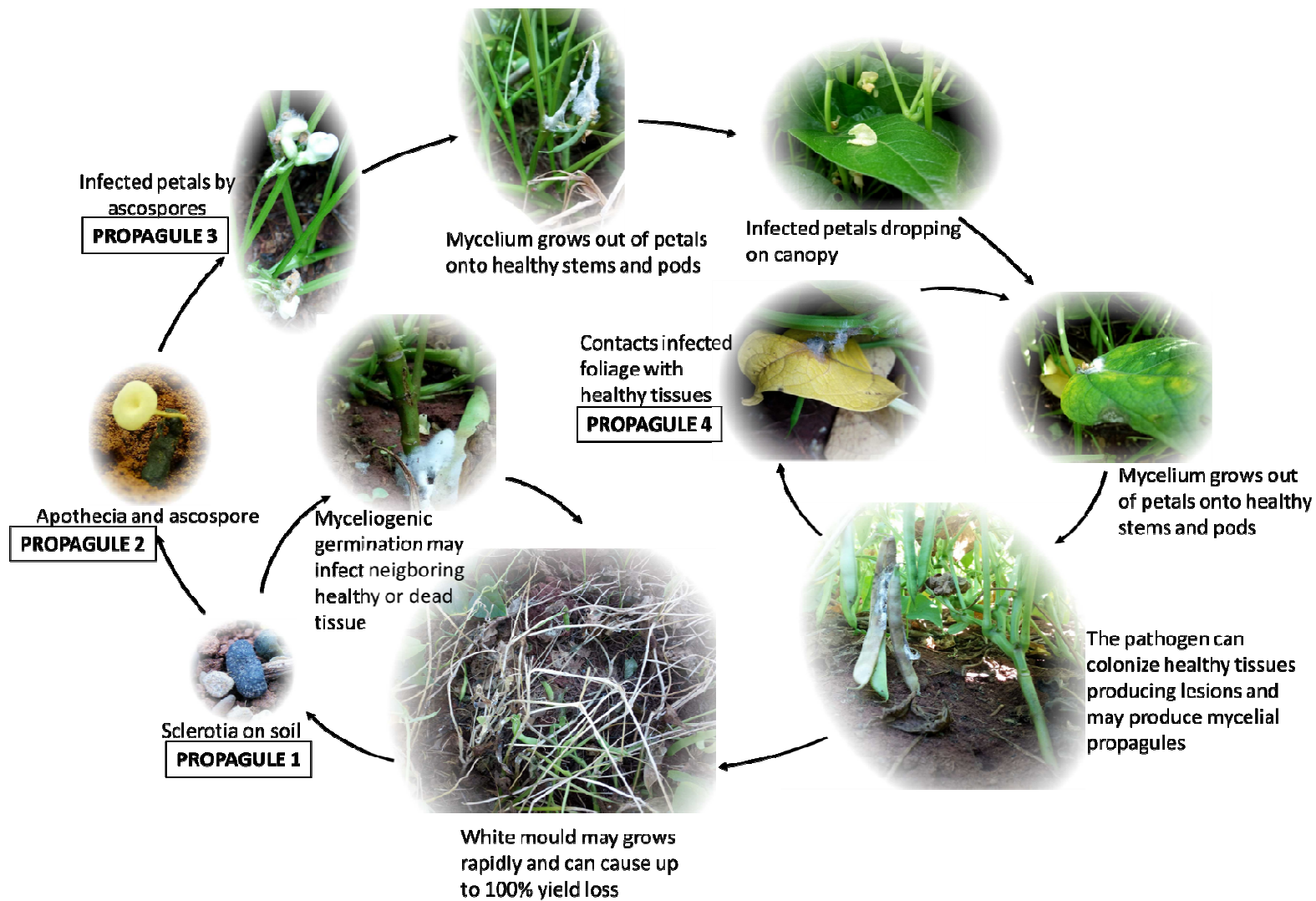
Figure 3. Life cycle of white mould disease (*S. sclerotiorum*) in *Phaseolus* beans showing disease propagules.
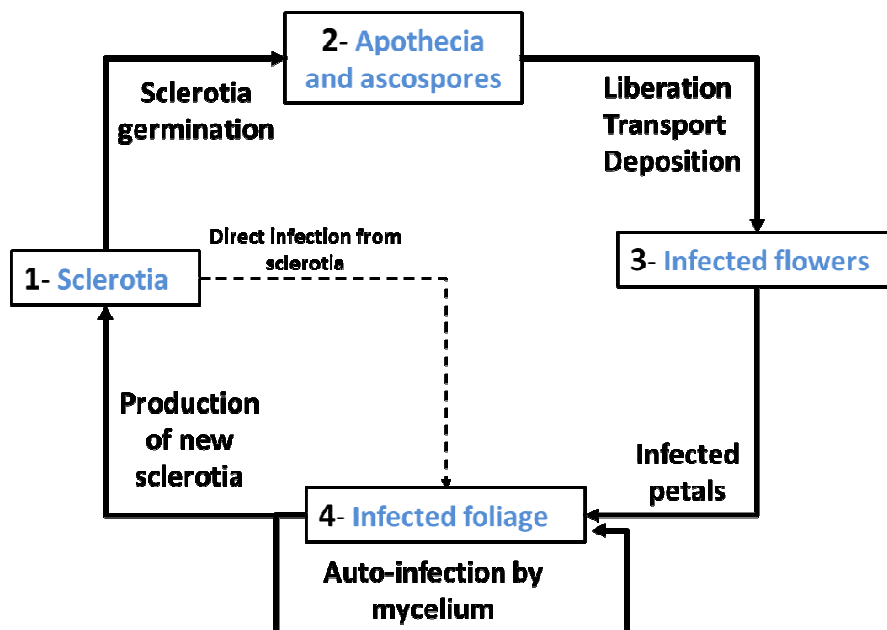
Figure 4. Generalized ethograph of white mould cycle as a function of the four disease cycle stages, where (**1-Sclerotia**) represents the amount of overwinter sclerotia, (**2- Apothecia and Ascospores)** is the number of apothecia and ascospores produced during the blooming period, (**3- Infected petals**) symbolize the number of infected petals during the blooming period and (**4- Mycelial spread**) characterizes mycelial contact and disease spread in vegetative host plant parts.
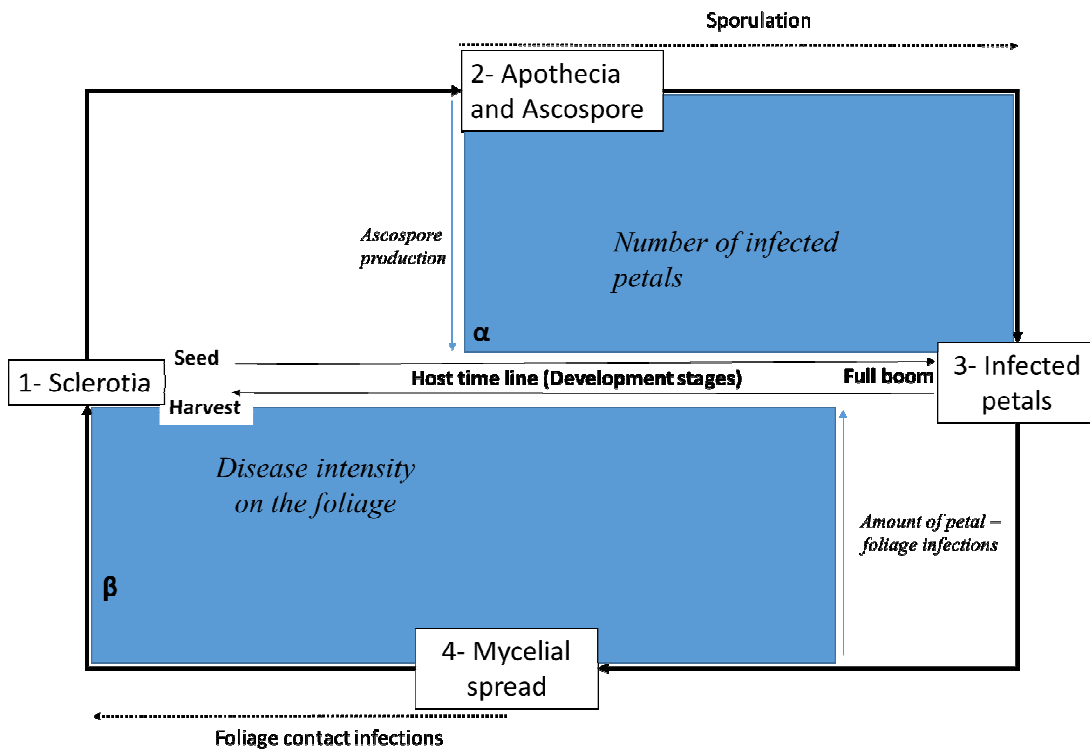
Figure 5. Generalized ethograph of the white mould disease cycle depicting relationships between propagules (and corresponding disease cycle stages) and host development stages. Ethograph of relationships between *S. sclerotiorum* propagules and bean development stages, where **1 (Sclerotia)** represents the amount of overwinter sclerotia, **2 (Apothecia and Ascospores)** is the number of apothecia and ascospores produced during the blooming period, **3 (Infected petals)** symbolizes the number of infected petals during the blooming period, and **4 (Mycelial spread)** characterizes hyphal contact and disease spread to vegetative plant parts. **Sporulation:** period of maximum ascospore release. **Ascospore production:** intensity of sclerotia carpogenic germination. **Amount of petal-foliage infection:** amount of foliage infected by petal infected. **Foliage contact infections:** amount of new infections caused by effective diseased and healthy tissue contact. **Blue square α** represents the number of infected petals primarily as a function of carpogenic germination and ascospore release, whereas **blue square β** represents the disease intensity on foliage as a function of the amount of foliage infections by petals and the amount of foliage contact infections.
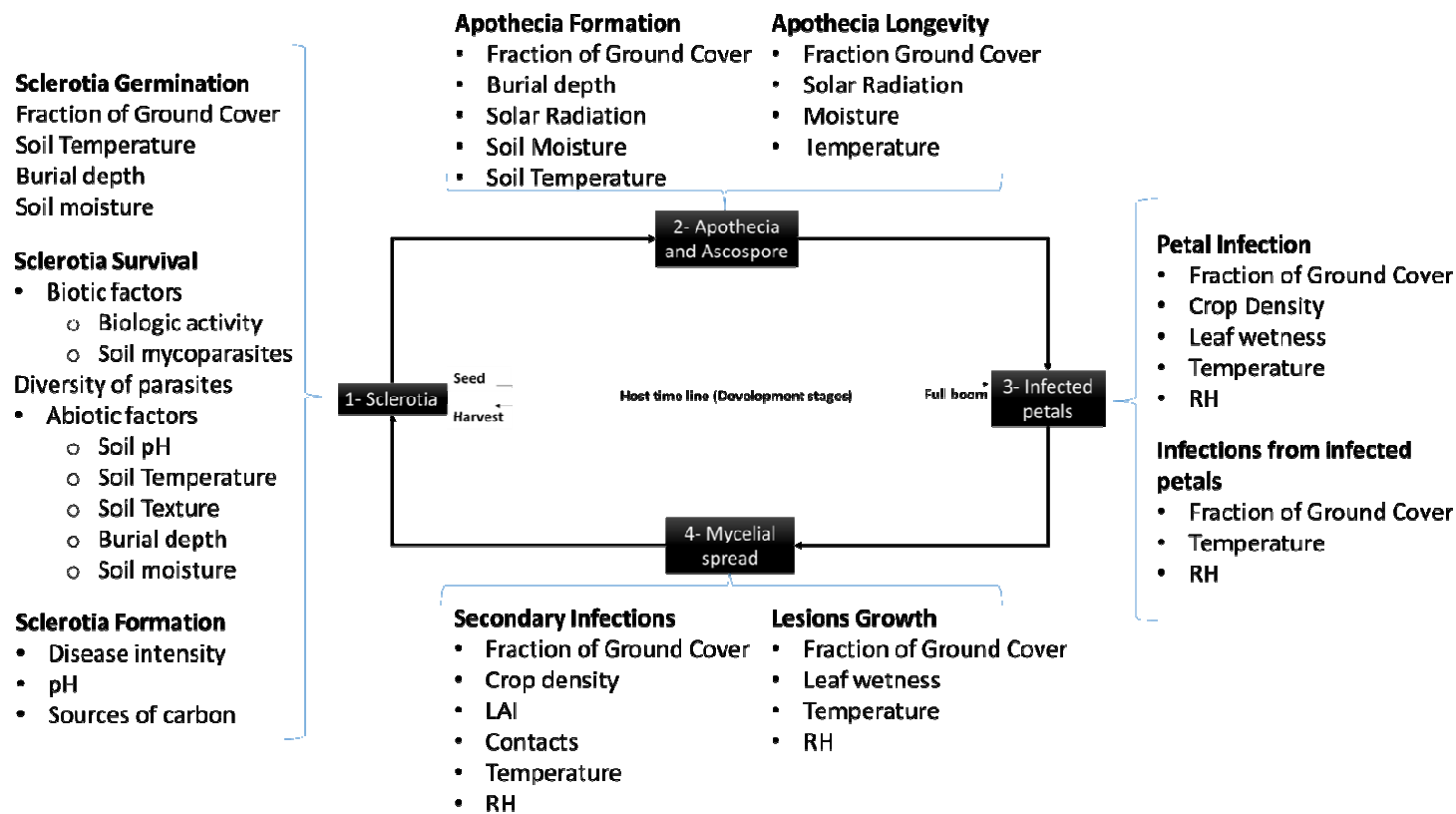
Figure 6. Generalized ethograph of white mould cycle showing four stages, subprocesses and their respective factors. (**1-Sclerotia**) represents the amount of overwinter sclerotia, (**2-Apothecia and Ascospores**) is the number of apothecia and ascospores produced during the blooming period, (**3- Infected petals**) symbolizes the number of infected petals during the blooming period, and (**4- Mycelial spread**) characterize mycelial contact and disease spread to vegetative plant parts.
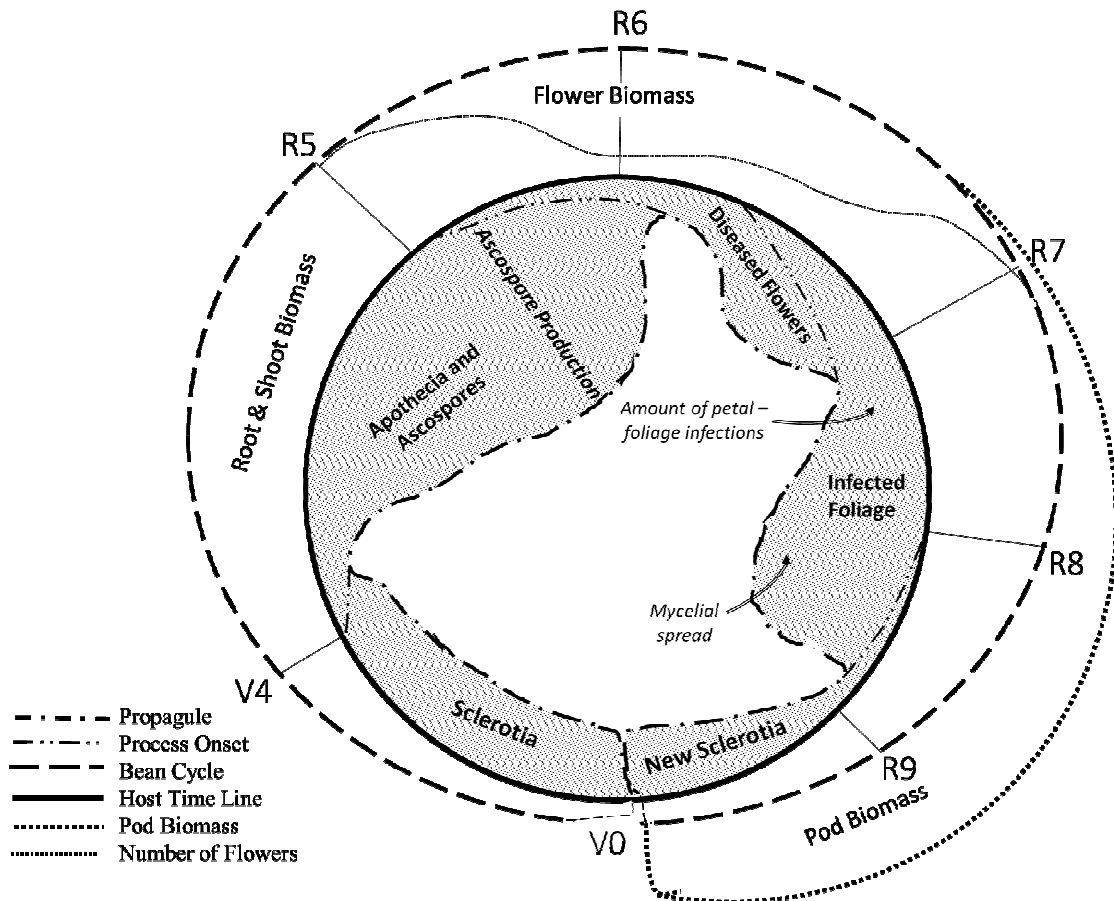
Figure 7. Phenomenology of white mould by propagule quantities and host development stages. Shaded area represents the population size of the pathogen in the form of sclerotia, apothecia/ascospores, fungal biomass in petals and fungal biomass in foliage. The area is relatively constant when propagules are mainly sclerotia, increases and decreases with the intensity of ascospore production, amount of petal-foliage infection, and infected foliage. **V0, V4, R5-R9** represent host development stages. **Ascospore production** starts at the Apothecia and Ascospores stage and leads to infected flowers. **Petal-foliage infection** occurs at the diseased flowers stage and leads to foliage infection. **Foliage contact infections** occurs in the infected foliage stage and new sclerotia are formed in the end of the host and disease cycles.

CHAPTER 2

EPIDEMIOLOGICAL PATTERNS AND DYNAMICS OF WHITE MOULD ON COMMON BEAN

EPIDEMIOLOGICAL PATTERNS AND DYNAMICS OF WHITE MOULD ON COMMON BEAN

ABSTRACT

This chapter analyses the successive phases of *Sclerotinia sclerotiorum* propagule, as well as their regulating processes and factors. Sets of experimental epidemics were created in bean fields under natural environmental conditions prevailing in the winter season in Central Brazil. The first (S1) and second (S2A) sets of epidemics were conducted in 2013 and 2014, respectively. The third epidemic set (S2B) was established in 2014. The sets of experimental epidemics aimed to promote a range of white mould epidemics. Fifty-seven epidemics, representing a wide variation in severity levels and in disease progress shapes, were monitored, with specific attention paid to the four considered propagule types. Epidemics were analysed by different statistical techniques, such as the study of scatterplots, temporal analysis, analysis of variance, regression, cluster and discriminant analyses, and temporal analysis. Three groups (A, B, and C) of epidemics were distinguished. Group A includes epidemics which were slow to establish, developed at a low rate, and led to very low terminal incidence and severity. Epidemics in Group C had an earlier onset, progressed very rapidly with an initially high rate of increase, followed by stabilization. Epidemics in Group B displayed an intermediate behaviour, with an initially low rate of disease increase, followed by a strong increase of the rate of foliage infection at later stages. A close relationship between incidence of diseased flowers and the number of lesions on foliage was found in Group C epidemics. The percentage of ground covered by the canopy (FGC) affected the number of white mould lesions from diseased flowers. An

interpretation of the results is that microclimatic conditions that prevailed in plots with varied FGCs altered the amount of disease associated and promoted a range of white mould epidemics. It is hypothesised that secondary infection rarely occurs in Group A epidemics because of the lower frequency of effective contacts, resulting in a small number of lesions on the foliage and low incidence of diseased plants (0.12). By contrast, it is hypothesised that, in epidemics of Group C, plant-to-plant spread occurs frequently, because of the higher frequency of effective contacts, leading to a much higher number of lesions and higher incidence of diseased plants (0.81). Group B includes epidemics with characteristics that are intermediate between Groups A and C. For bean white mould epidemics, the proportion of plants that become diseased depends on the frequency of effective contacts between host and propagules. Therefore, even if the primary inoculum in the form of sclerotia and the subsequent formation of apothecia are, in part, relevant, the role of subsequent infections by other propagules on white mould epidemics must be taken into account.

1. INTRODUCTION

White mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a main cause of yield losses of common beans (*Phaseolus vulgaris* L.) in all world bean growing regions (Ramasubramaniam et al., 2008). Brazil is one of the main *Phaseolus* bean growers wordwide, with an annual yield of *c*. 3.1 million tons in an area of *c*. 3.0 million ha (FAO, 2015). The crop is traditionally grown in three cropping seasons per year, known as rain-season crop, dry-season crop and winter-season crop (Oliveira et al., 2013). The irrigated winter bean crop, 28.7 % of the annual crop in 2014 (CONAB, 2015), is characterized by dry weather and mild temperatures (*c.* 20°C), and is the bean crop most affected by white mould. Typically, Brazilian bean cultivars are of indeterminate growth, with prostrate habit, which are conducive to white mould epidemics (Napoleão et al., 2005).

The life cycle *of Sclerotinia sclerotiorum* is complex and its epidemiology in common beans is somewhat unique. From an epidemiological viewpoint, it can be divided into four broad phases, each corresponding to one functional propagule. The first phase corresponds to the process of carpogenic germination of the soil sclerotia (propagule 1), which is the key event in disease onset, as it triggers the growth of apothecia (Adams & Ayers, 1979, Purdy, 1979, Sun & Yang, 2000). In the second phase, ascospores (propagule 2) are dispersed from apothecia into the air. Ascospores are considered another source of inoculum, when infecting bean flowers during host blooming stage (Mclean, 1958, Newton & Sequeira, 1972, Abawi & Grogan, 1975). Flowers or individual petals provide a suitable nutrient base for the initial colonization following ascospore infection (Sutton & Deverall, 1983). These infectious mycelium in petals (propagule 3) constitute the third phase of the bean white mould cycle, which in

turn will spread the disease to the foliage and other vegetative plant parts: infected petals are dislodged and dispersed onto the foliage, where they may initiate infections. Thus, primary infection on vegetative parts is generated by infected petals (Mclean, 1958, Abawi & Grogan, 1979). The fourth phase corresponds to the secondary infection on foliage, which may develop from spread of mycelium (infectious hyphae, propagule 4) from diseased tissues to healthy ones, similar to the process taking place in "compound interest diseases" *sensu* (Van der Plank, 1963).

The successive types of propagules, and the processes through which these types of propagules are generated have not been considered in detail in the bean - white mould pathosystem. This is in part probably due to the incorrect association which has been made between soil-borne plant pathogens and the so-called monocyclic ("simple interest") epidemics. Pfender (1982) discussed this misconception and highlighted that Van der Plank´s notion of monocyclic and polycyclic diseases was proposed on the basis of the biological dynamics of the pathosystem and not on the shape of disease progress curves. Van der Plank distinguished two types of epidemics based on the features of the disease cycle: in "compound interest" epidemics, the inoculum produced in infected plant units serve as sources for additional, new, infections in the same season (therefore, the pathogen moves from lesion to lesion or from plant to plant). By contrast, in "simple interest" epidemics, one (infected) plant unit does not infect another plant unit in the same growing season (Van der Plank, 1963).

The different phases of the life cycle of *S. sclerotiorum* have been described and studied separately by many authors. For instance, the effects of soil temperature, moisture, and burial depths on carpogenic germination *of S. sclerotiorum* have been studied (Mitchell & Wheeler, 1990, Ferraz et al., 1999, Wu & Subbarao, 2008). Other

authors studied the effects of environmental factors on the survival of ascospores (Caesar & Pearson, 1983). However, an analysis of the mechanics of white mould epidemics, where the four phases of the pathogen life cycle are concatenated, has not been attempted.

The present study analyses the effect of different phases of the life cycle of the pathogen, together with the processes and factors involved in the disease cycle in sets of epidemics varying greatly in intensity under natural field conditions. This population of white mould epidemics was then analysed to highlight the importance of each of the four types of propagules.

2. MATERIALS AND METHODS

A series of field experiments were conducted, where the bean – *S. sclerotiorum* pathosystem was manipulated, with different fraction of ground covered and doses of sclerotia, so that white mould epidemics of widely different intensities and shapes developed.

2.1 Experimental location, crop establishment and inoculum

Experimental areas were planted with bean cv. Pérola (indeterminate growth habit), and were conducted so as to generate epidemics of widely different intensities. The crops were grown during two time periods. Two sets of epidemics were studied from May to September in 2013 (early winter, sowing date: May 10th, 2013) and one epidemic set was studied from June to September in 2014 (winter/spring, sowing date: June 23rd, 2014). These three successive, or synchronous (season 1) but distinct (season 2) experiments are referred to as 'epidemic sets'. Experiments were performed on a 1 ha field area of the EMBRAPA Rice and Beans Experimental Station,

located at Goianira, State of Goiás (coordinates: 16º 26' 09.2" S, 49º 24' 06.8" W; 735 m a.s.l.). The soil was classified as clay latosol, with pH 6.6. The temperature ranged from 8.6 and 34.9°C with an average of 22.7°C (AGRITEMPO), which is conducive to white mould epidemics (Hannusch & Boland, 1996).

Beans were planted with an inter-row spacing of 0.50 m and a seed density of 240,000 seeds.ha^{-1} in all epidemic sets. One application of 300 kg.ha^{-1} of mono-ammonium phosphate (9% of N and 48% of P_2O_5), based on previous soil analysis, was done at sowing. One seed dressing was applied with 50 kg.ha^{-1} of N, 25 days after sowing. A sprinkler irrigation system provided favourable conditions for crop growth and white mould development. Irrigations (15 mm.m^{-2}) were done every three days.

2.2 Origin of primary inoculum, sclerotia density estimation and sampling

The area chosen for conducting all trials had been used for five years for white mould studies and therefore, was already infested with sclerotia of *S. sclerotiorum*. In addition, before planting each set of experiments, fresh inoculum of *S. sclerotiorum*, in the form of sclerotia, was applied evenly onto the soil surface, aiming to achieve the most uniformly-distributed distribution of viable inoculum according to treatments described in section 2.3.1. Sclerotia used for infestation were obtained in March, 2013, from a seed-processing unit, located in Silvânia County, State of Goiás, where sclerotia are separated from soybean residues during the cleaning process. Collections of sclerotia were stored at *c*. 25 ° C in large plastic bags (500 kg) until use in 2013 and 2014. One single batch of inoculum was used to infest soil in all set of epidemics.

After calibration, a manual fertilizer dispenser (acme Am-s18®), was used for the homogeneous distribution of sclerotia. Immediately after inoculum distribution,

samplings were conducted to verify if each designated density had been satisfactorily accomplished. In some instances, one additional distribution was made to ensure that the amount of initial inoculum designated for the plot was achieved. Sclerotia sampling was carried out in 1 m^2 in each plot. *Sclerotia density* (SC) in a microplot refers to the amount of sclerotia per square meter observed on the soil surface after sowing.

2.3 Field experiments

2.3.1 Inoculum levels for sets of white mould epidemics

The first (S1) and second (S2A) sets of epidemics were conducted in 2013 and 2014, respectively. These sets of epidemics were all carried out with a single nominal inoculum level, and variations in the dynamics of the disease were therefore studied on this fixed level. Therefore, both S1 and S2A consisted in a single nominal inoculum treatment, with an average of 200 sclerotia per square meter. Sclerotium distribution was carried out immediately after crop sowing.

The third epidemic set (S2B) was established in 2014. Plots of this set were established in the same infested area which had been used in experiment S1 in 2013. A variable number of nominal inoculum levels were created in this set of epidemics. Six initial inoculum densities, corresponding to six doses of sclerotia (0, 10, 50, 100, 200 or 400 sclerotia.m^{-2}) were applied to soil soon after sowing. In this set of epidemics, inoculation was also carried out soon after sowing.

Epidemic set S2B included control plots, where no additional inoculum had been added (0 sclerotia.m^{-2}). To further suppress primary inoculum on soil from previous year in these control plots, these plots were treated with fluazinam (Frowncide® 100 ml.ha^{-1}), applied to the soil surface. The fungicide was applied with a bar sprayer with

four full cone nozzles, spaced 50 cm apart, connected to a CO_2 cylinder giving a spray volume of 200 L.ha^{-1} at 2.6 bar. This application inhibits the carpogenic germination of sclerotia.

2.3.2 Statistical design and experimental lay-out of the epidemic sets

Each set of epidemics was created in an area of 150 x 50 m (Annex 1). Epidemic sets S1 and S2A, which involved a single nominal dose of initial inoculum (200 sclerotia.m^{-2}), were performed in 16 and 17 individual epidemics, respectively, each of them representing a given variation about this nominal dose. In epidemic set S2B, each treatment (0, 10, 50, 100, 200 or 400 sclerotia.m^{-2}) was replicated four times, totalling another 24 white mould epidemics. One of the epidemics of set S2B was excluded from analyses because the respective disease data had been lost. Each plot was 48 m² in area, with sixteen 6-m lines of bean plants, and was separated from each other by 1-m uninoculated bean lines and minimal distance of 8 m between microplots to avoid interplot interference. Broers and Lopez-Atilano (1995) argued that if novel airborne infections are not important for disease development, the effect of inoculum exchange among plots will be negligible, and consequently the level interplot interference will be minor. Therefore, the epidemics are considered independent. Disease variables were measured on microplots (2 x 2.5 m) (Annex 1) that were defined at the centre of each plot (see below).

2.3.3 Characteristics of microplots

Disease level microplots for epidemics sets S1 and S2A were randomly distributed in the field, defined at flowering onset, along with the initial disease evaluations. In epidemic set S2B, plots were distributed into four blocks according to

the topography of the experimental area, to reduce experimental error. Each microplot represented one independent epidemic and was composed of six 2-m rows of bean plants, and a total area of 5 m² (annex 1). Microplots were subdivided into 10 quadrats of 0.5 m², corresponding to the area between rows of 1 m in length (Annex 1). The division of microplots in quadrats enabled more reliable counts of apothecia and of new sclerotia formed at end of season.

2.4 Observations

The list of symbols, dimensions and units of the experimental variables are described in Table 1.

2.4.1 Estimation of apothecia density

The assessment of the variable *density of apothecia* (APTOT) was initiated 52 days after sowing (DAS) and performed daily up to the end of the flowering period (68 DAS) by visual counts of apothecia observed on the ground. To improve evaluations, counts were done in each of the ten quadrats of the microplots. The sum of the number of apothecia in all quadrats is the estimate of APTOT.

2.4.2 Incidence of diseased flowers and detection of *S. sclerotiorum*

Flowers were sampled at three different layers of the bean canopy to estimate the *incidence of diseased flowers* (IDF), which corresponds to the incidence of flowers infected by *S. sclerotiorum* on full bloom period. The three layers correspond to flowers in the upper canopy, flowers within the canopy, and flowers dropped on the ground. Thirty flowers were collected per microplot, ten flowers in each layer. Collected flowers were directly placed in 1.5 ml Eppendorf tubes and stored at -20°C for 30 days until plating. Flowers were considered infected when a yellow halo was

formed around petals plated on Neon medium, indicating the presence of *S. sclerotiorum* (Napoleão et al., 2006). The proportion of diseased flowers in a microplot was estimated as the mean of the proportions of diseased flowers in the three layers considered.

2.4.3 Operational definition of a lesion on the foliage and plant disease incidence assessment

Assessments of white mould incidence on the foliage accounted for the fact that symptoms caused by *S. sclerotiorum* on bean vary with the stages of lesion development. In the early stages of lesion development the foliage shows light pale to dark brown discoloration. Subsequently, the lesions are often covered by rapid growing white cottony fungal mycelium. For incidence assessments on plants, each bean plant was considered infected when foliage or stem displayed discoloured lesions on foliage or typical white cottony mycelium. Disease incidence was estimated as the proportion of diseased plants assessed on all 40 plants of the two central rows of a microplot. In epidemic sets S2A and S2B, the first incidence assessment was done at 52 DAS and successive assessments were performed once a week up to 82 DAS, totalling five assessments (I_1 to I_5). In epidemic set S_1, incidence assessments started at 68 DAS and were performed weekly up to 82 DAS, totalling three assessments, corresponding to assessments I_3, I_4, and I_5 of experiments S2A and S2B.

2.4.4 Quantification of number of white mould lesions on foliage

At the end of the blooming period for cv. Pérola (68 DAS), the number of white mould lesions per microplot (*lesion density,* NL) was estimated on all 40 plants on the two central rows of the microplot. The number of lesions per microplot corresponds to

the sum of lesions on all 40 plants. For NL assessments, typical symptoms, such as individual foliage lesions or white cottony mycelium growth were counted as one lesion. Number of lesions was used to estimate the amount of injuries arising from flower infections.

2.4.5 White mould severity assessment

White mould *severity* (S) is the proportion of diseased foliage in the two central quadrats of a microplot, assessed through a disease severity rating scale. The disease scale consists of seven disease severity classes, where grade 1 corresponds to the absence of disease symptoms; grade 2 corresponds to 1% to 5% of the microplot area with white mould symptoms (AWS), grade 3 from 6% to 20% of AWS, grade 4 from 21% to 50% AWS, grade 5 from 51% to 70% AWS, grade 6 from 71% to 90% AWS, and grade 7 from 91% to 100% of diseased area (Geraldine et al., 2013). In epidemic sets S2A and S2B, the first severity assessment was done at 52 DAS and was performed weekly up to 82 DAS, totalling five assessments, S_1 to S_5. In epidemic set S_1, severity assessments started at 68 DAS and were performed weekly up to 82 DAS, totalling three assessments, corresponding to severity assessments S_3, S_4 and S_5.

2.4.6 Estimation of fraction of ground covered

The *fraction of ground cover* (FGC) refers to the proportion of ground covered by the bean crop canopy at the end of flowering (68 DAS). Two scores were given for each microplot, one score for quadrat 5 and one score for quadrat 6 (Annex 1) to derive an average value for FGC. The fraction of ground covered by the plant canopy was scored using a scale of 1 to 5 adapted from Deshpande (1992), where:

- 1: open canopy with ground surface between rows completely visible;

- 2: open canopy with ground surface between rows up to 70% visible;

- 3: open canopy with ground surface between rows up to 40% visible;

- 4: closed canopy but with ground surface between rows up to 10% visible;

- 5: completely closed canopy over the furrow, no ground surface visible.

2.4.7 Estimation of newly formed sclerotia

The *density of new sclerotia* (NS) refers to the quantity of new sclerotia per square meter (NS) formed at the end of the white mould epidemics. NS was estimated after harvest at 100 DAS. New sclerotia may be formed either internally in the pith of the stem, or on the external plant surface. Therefore, the amount of new sclerotia formed was quantified in two steps, by the sum of sclerotia collected on the ground and sclerotia retrieved from plant residue. A first assessment was carried by sieving plant residues through a 5 mm–pore sieve. A second assessment was derived from the number of sclerotia counted at the soil surface after harvest. This assessment was performed in the two central quadrats of the microplot (Quadrats 5 and 6 , totaling 1 m^2). Sclerotia per square meter of microplot were estimated by the sum of sclerotia numbers obtained on these two steps. Old sclerotia are light gray and covered with clay particles. The new sclerotia were clearly distinguishable from old ones by their intense dark colour and lack of clay particles on surface.

2.5 Analyses of the white mould epidemics

2.5.1 Data Processing

All results obtained in the 56 epidemics (16 epidemics in S1, 17 in S2A and 23 in S2B) were analysed statistically. Disease progress in each microplot was treated as an

independent epidemic. The minimum distance of 8 m among microplots helped warrant the independence of epidemics.

2.5.2 Temporal analysis of disease progress curves

Incidence and severity progress curves were constructed from the five successive assessments of incidence and severity between 52 DAS and 82 DAS. The list of symbol, dimensions and units of disease progress curve variables is given in Table 1. Following the construction of disease incidence progress curves, the following variables were estimated:

lowest non null value of disease incidence (Imin);

maximum incidence observed (Imax);

maximum slope of incidence progress curve (Islope);

area under incidence progress curve (AUIPC); and

relative area under incidence progress curve (RAIPC).

The subsequent variables were calculated from the disease severity progress curves:

lowest non null value of disease severity (Smin);

maximum severity observed (Smax);

maximum slope of the severity progress curve (Sslope);

area under severity progress curve (AUSPC); and

relative area under severity progress curve (RASPC).

Temporal analysis was carried out according to the methodology described by Campbell and Madden (1990). Equations with linear parameters from each of two models, *i.e.* monomolecular ($\ln [1/(1-y)] = \ln [1/(1-yo)]+r_M t$) and logistic ($\ln [y/(1-y)]=$

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ln [yo/(1-yo)]+r_Lt), were employed as prediction equations to compare linearly transformed data and thus the "adjusted" coefficient of determination (R^{2*}) was estimated for each simulated epidemics. The coefficients of determinations (R^{2*}) were estimated for each progress curve model (monomolecular or logistic) in each white mould epidemics with SYSTAT®, Version 13.0 (SYSTAT® Software, Inc., Chicago, IL). The Euler ("rectangle") method rule (Campbell & Madden, 1990) was used to calculate the area under incidence and severity progress curves. These analyses were performed with 39 white mould epidemics, which incidence assessments were performed five times.

2.5.3 Scatterplots and regression analyses

Scatterplots were drawn and regression analyses were conducted to visualize and assess the relationships between pairs of variables. In these graphs, several variables of the disease cycle were considered, and paired: SC x APTOT, APTOT x IDF and IDF x NL. These analyses were performed with SYSTAT®, Version 13.0 (SYSTAT® Software, Inc., Chicago, IL).

2.5.4 Cluster analyses

Hierarchical cluster analysis using a Ward criterion and the Mahalanobis distance was performed to classify white mould epidemics into allied groups. The Mahalanobis distance was chosen as the cluster metric because: (i) variables are quantitative, with (ii) differing ranges of values (Wilkinson et al., 2007). A preliminary cluster analysis was performed first, using all variables related to the disease progress curves (I_3, I_4, I_5, Islope, Imin, Imax, RAIPC, S_3, S_4, S_5, Sslope, Smin, Smax, RASPC; Table 1). A second cluster analysis was performed to derive groups that would be similar the groups

derived in the preliminary cluster analysis, but with a reduced number of variables. Lastly, cluster analysis was performed on incidence and severity variables only (I_3, I_4, I_5 S_3, S_4, S_5). Hierarchical cluster analysis was performed with SYSTAT®, Version 13.0 (SYSTAT® Software, Inc., Chicago, IL).

2.5.5 Discriminant analysis

A discriminant analysis involving variables characterizing the 56 epidemics was performed to study the relationships between a dependent, qualitative, variable (*i.e.*, whether a given epidemic belongs to one of the three epidemic groups A "low incidence", B "moderate incidence" and C "High incidence"), and a set of assumed independent microplot variables (FGC, SC and APTOT) that quantitatively characterize the conditions under which epidemics were initiated. Discriminant analysis was also performed with SYSTAT®, Version 13.0 (SYSTAT® Software, Inc., Chicago, IL). Discriminant analysis aimed to identify the main variables that characterized the epidemic groups separated by the hierarchical cluster analysis. This analysis establishes weights of the dependent variable for each independent variable, aiming to maximize the variance between groups, consequently reducing the variance within groups (Engelman, 2009).

2.5.6 Analyses of variance

Univariate statistical analysis of variance was performed with the SAS® 9.1 statistical package (SAS® Institute, Cary, NC, USA). The GLM procedure was run to assess the grouping effect on experimental variables and disease progress curve variables. To compare the groups, a joint analysis was performed (GLM procedure) with a random model, where groups were considered as random effects. The groups

from hierarchical cluster analysis should be considered random. The analysis of variance (α = 0.05) was supported by the Shapiro-Wilk test for the normality of residuals, Leneve test for homogeneity of variance, and the Durbin-Watson test for independency of residues and to check for outliers. To meet the assumptions of residuals normality and the homogeneity of variance, Islope, Imin, Imax, RAIPC, Sslope, were adjusted using a square root transformation, while APTOT, SC, NS, SMAX and RASPC were transformed using individual Box-Cox transformation (Box & Cox, 1964).

3. RESULTS

3.1 Relationship between types of propagules

Scatterplots showed strong positive association between the main stages of the disease cycle (Figure 1), represented by the initial inoculum (SC), the number of apothecia that had been produced (APTOT) and which released ascospores leading to flower infections (IDF), and which in turn led to lesions on the foliage (NL). A first positive association was found for SC and APTOT (Figure 2). Another positive relationship was detected between APTOT and IDF: when the amount of APTOT is high, IDF is also high (Figure 2). A third positive association between IDF and NL was also observed (Figure 2). Lastly, as expected, an increase in NL is associated to high incidences and severities of white mould (Table 4). It is worth noting that, although significant ($P < 0.01$) in all but one case, these pairwise associations appear to differ in strength: while the IDF-NL and the APTOT-IDF associations appear to be strong, the SC-APTOT and the SC-IDF associations appear weaker, and the SC-NL association is not significant (Figure 2).

3.2 Clustering of white mould epidemics

A parsimonious use of the epidemiological characteristics, that is to say, the sole use of disease incidence and severity data (I_3, I_4, I_5, S_3, S_4, and S_5) for classification purposes led to identify three distinct groups from hierarchical cluster analysis among 57 white mould epidemics (A, B and C) (Figure 3). Epidemic groups A, B, and C gathered 24, 11, and 22 white mould epidemics, respectively. This last grouping with further reduced number of variables was 87.7% similar to the first grouping with all 14 variables, based on the number of epidemics that were placed in the same group.

3.3 Analyses of variance

Analysis of variance did not indicate significant differences among the three epidemic groups with respect to density of sclerotia (P = 0.222) (Table 2). On the other hand, the differences among groups originate (at least in part) from differences in APTOT (P < 0.01).

Incidence of diseased flowers (IDF) and fraction of ground cover (FGC) also showed statistically significant differences (P < 0.01) between epidemic groups (Table 2). In addition, analysis of variance of disease incidence curve variables indicated that the three clusters were significantly different in Imin (P < 0.01), Imax (P < 0.01), Islope (P < 0.01) and RAUIPC (P < 0.01). The same holds true for disease severity variables, such as SMIN (P < 0.01); SMAX (P < 0.01); Sslope (P < 0.01); and RASPC (P < 0.01) (Table 3).

3.4 Characterization of groups

Characteristics of epidemic group A: Group A is characterised by incidence and severity progress curves with disease onset at *c*. 60 days after sowing and a constant and small slope of disease progress curves (Figure 4d). Both the average incidence and

the average severity progress curves increase according to a simple linear shape (Figure 4d; Annex 3).

With respect to the number of sclerotia per square meter, group A has an average SC value of 150.8 sclerotia.m^{-2} (Figure 4a), which is not significantly different from the SC values of group B or group C (Table 2). However, group A has the lowest apothecia density (APTOT) value, with 45.4 apothecia per microplot or 9.1 apothecia.m^{-2} (Figure 4a). With respect to the incidence of diseased flowers, group A has an average IDF value of 0.24 or 24.5% diseased flowers (Figure 4a). Consequently, this group has the lowest lesion density (NL) (7.27 lesions per forty plants) and also lowest density of new sclerotia (NS) with 109 new sclerotia per square meter (Table 4). When considering the fraction ground cover, group A has the lowest FGC value compared with the other epidemic groups, (average 2.29, over a scale of 1 to 5, Figure 4a). Regarding the disease progress curve, group A is characterized by non-null incidence value (IMIN) of 0.06 or 6% of diseased plants per microplot (Figure 5d).

Group A is also characterized by a maximum incidence (IMAX) value of 0.12 or 12% of diseased plants per microplot (Figure 5g; Table 5) the lowest maximum slope of incidence progress curve (Islope = 0.01) (Figure 5a; Table 5) and the lowest relative area under incidence progress curve (RAIPC = 2.71 diseased plant.day) (Figure 5c). This group also presented a SMIN (lowest non null severity) mean value of 0.01 or 1% of diseased foliage per microplot (Figure 5e) and a SMAX mean value of 0.04 or 4% of diseased foliage per microplot (Figure 5h). With respect the maximum slope of severity progress curve (Sslope), group A is associated with a mean value of the relative rate of disease increase of 0.003 foliage. foliage^{-1}.day^{-1} (Figure 5b). When considering the

relative area under severity progress curve (RASPC), group A has mean value 0.55 diseased foliage.day (Figure 5f).

Characteristics of epidemic group B: Disease progress curves of group B show a regular increase until the last assessment (82 DAS) (Figure 4e; Table 5; Annex 4). Group B is characterized by a variable slope of the incidence and severity progress curves (Figure 4e). Group B displayed a larger rate of disease incidence at 68 DAS than group A (Figure 4e). The terminal disease severity was approximately five times higher in the epidemics of group B than in epidemics of group A (*c.* 17% compared to *c.* 3.6%). When considering the number of sclerotia per square meter, group B has a mean SC value of 151.8 sclerotia.m^{-2} (Figure 4b), which is however not statistically different from group A or group C (P = 0.22) (Table 2).

When considering number of apothecia per microplot, group B is not significantly different from group A with 60.5 apothecia per microplot or 12.1 apothecia.m^{-2} (Figure 4b). Nevertheless, with respect to the incidence of diseased flowers, group B has an average IDF value of 0.324 or 32.4% of diseased flowers (Figure 4b), lower than group C (P < 0.01) (Table 4). Regarding the fraction ground cover, group B has higher FGC score than group A with 3.14, over a scale of 1 to 5 (Figure 4b). Thus, this group has an average lesion density (NL) of 22.38 lesions per forty plants) and an average density of new sclerotia (NS) of 364 new sclerotia per square meter (Table 4). This group has higher NS than group A.

There was a significant difference between group B and the other groups of epidemics with respect to the Imin (non-null incidence, mean value of 0.23; Table 5). This means that there are 23% of diseased plants per microplot (Figure 5d). When

considering the maximum slope of the incidence progress curve (Islope), group B also has a higher Islope value than group A with a mean value of 0.04 (*P* < 0.01) (Figure 5a; Figure 4b). This group is also characterized by a higher maximum incidence value than group A, with a mean value of 0.48 or 48% diseased plants per microplot (Figure 5g).

With respect to the mean of relative area under incidence progress curve, group B has a lower RAIPC value than group C with value of 8.74 (*P* < 0.01) (Figure 5c; Figure 2B.3.4; Table 5). Group B is associated with SMIN value lower than group C (SMIN = 0.03 or 3% diseased foliage per microplot vs. 0.08 or 8% on group C) (Figure 5e). Group B was also characterised by a higher Sslope (mean value of 0.02) than group A (Figure 5b) and by a lower maximum severity value than group C (SMAX = 0.18 or 18% diseased foliage per microplot) (Figure 5h). When considering the mean relative area under severity progress curve, group B exhibited a lower RASPC value than group C with RASPC value of 2.01 (Figure 5f). Group B exhibited a higher RASPC value than group A.

Characteristics of epidemic group C: Epidemics belonging to group C showed severity progress curves that increased according to a simple linear shape after full bloom period, but with a strong progress rate (Figure 4f). With respect to the incidence progress, group C presents a clear sigmoid-shaped curve (Figure 4f; Annex 5). The slope of the disease incidence progress curve at the onset of epidemics was the highest among all three epidemic groups (Figure 4f). The terminal incidence was almost seven times higher in group C than in epidemic group A (*c.* 81% compared to *c.* 12%) and 1.7 times higher than in group B epidemics (81% *vs.* 47%). Terminal disease severity in group C was approximately three times higher than in group B (*c.* 54% compared to *c.* 17%).

After full bloom (68 DAS), however, disease incidence increases at a much higher rate in group C (approximately twice as high) than in group B. With respect to the number of sclerotia per square meter, group C has the highest value, with 197.7 sclerotia.m^{-2} (Figure 4c), not different from group B or A (P = 0.22) (Table 2). However, group C displayed a higher apothecia density value than group A (APTOT = 145.5 apothecia per microplot or 29.1 apothecia.m^{-2})(Figure 4c). With respect to the incidence of diseased flowers, group C presented the highest IDF value of 0.575 or 57.5% of diseased flowers (Figure 4c). When considering the fraction of ground cover, group C also had the highest score of all groups (3.82) (Figure 4c). Consequently, this group has the highest lesion density (NL) (80.52 lesions per forty plants) and also highest density of new sclerotia (NS) with 736 new sclerotia per square meter (Table 4).

Epidemic group C has a higher Imim value (0.24) than group A (Figure 5d). Group C presents the highest Islope, differing significantly from the other epidemic groups, with 0.06 plant. plant^{-1}.day^{-1} (Figure 5a). This group also has the highest maximum incidence value (IMAX= 0.81 plant.plant^{-1} or 81 % of diseased plant per microplot) (Figure 5g). Epidemic group C has highest RAIPC value than others epidemic groups, with 15.5 diseased plant.days. (Figure 5c).

Group C is characterized by the highest non null severity (SMIN) value, with 0.08 plant.plant^{-1} (Figure 5e; Table 5). This epidemic group is also characterised by the highest Sslope value (mean value of 0.04 foliage.foliage^{-1}) (Figure 5b) and the highest maximum severity value (SMAX = 0.55 or 55% diseased foliage per microplot) (Figure 5h, Table 5). When considering the mean of relative area under severity progress

curve, group C has a higher RASPC value than epidemic group A and B, with 7.48 foliage.foliage^{-1}.day (Figure 5f).

3.5 Discriminant analyses

The discriminant function correctly characterized 67% of epidemics actually belonging to group A (Figure 6), *i.e.*, 16 epidemics were correctly classified in group A, while seven epidemics were incorrectly classified in group B and one epidemic was incorrectly classified in group C (Table 6). Regarding group B, the discriminant function correctly characterized 56% of the epidemics actually belonging to group B (Table 6). As to group C, the discriminant function correctly characterized 73% of the epidemics belonging to this set (Figure 6), *i.e.*, sixteen epidemics were correctly classified in group C, while four epidemics were incorrectly classified in group B and two epidemics were incorrectly classified in group A (Table 6).

When considering Jackknifed classification (Engelman, 2009), three variables, *vis*. fraction of ground cover (FGC), apothecia per microplot (APTOT) and sclerotia per square meter (SC) correctly characterized 67% of the three epidemics groups (Table 6). The Wilks's Lambda statistic has been used for the multivariate test of dispersion among all the groups on all the variables. Lambda varies from 0 to 1, with 0 meaning that group means differ, and 1 implying that all group means are the same. A Lambda value of 0.345 was found, indicating that group means differ ($P < 0.01$).

3.6 Temporal analysis of white mould epidemics

The temporal analysis of white mould incidence in group A showed 10 of 12 epidemics with a better fit for the monomolecular model (Table 8) with coefficients of determination (R^2) ranging from 63.2% to 94.8% (Annex 2). On the other hand, in

seven out of 11 white mould epidemics of group B, the logistic model had a better fit for disease progress curves (Table 8). These seven epidemics had R^2 that ranged between 77.3% and 95.7% (Annex 2). In group C, the logistic model had a better fit to disease progress curves in nine of 16 epidemics (Table 8) with R^2 values between 88.7% and 99.8% (Annex 2).

4. DISCUSSION

This study highlights a series of features that characterize white mould epidemics in *Phaseolus* bean fields based on the analysis of fifty-seven widely different epidemics. I mainly discuss and analyse the relevance of primary and subsequent infection processes, born from successive types of propagules, in the bean-white mould pathosystem which has usually not been considered in most of studies.

The independence of epidemics was ensured by several factors pertaining either to disease characteristics or to experimental conditions. The first factor is related to the strong spatial aggregation of white mould on beans, where epidemics usually are dominated by localized sources of inoculum (Jones et al., 2011), which implies reduced exchange of inoculum among microplots. The fragile ascospores of *S. sclerotiorum* survive poorly during air transport, and viability is short (Caesar & Pearson, 1983). In addition, the effect of inoculum exchange on the epidemic and the level of interplot interference is small when the new infections are relatively unimportant for disease development (Broers & Lopez-Atilano, 1995). Finally, the extremely variable range of shapes of the disease progress curves of epidemic groups A, B and C attest that each individual epidemic developed independently of the others.

Van der Plank (1963) distinguishes between two types of disease epidemics. He uses the notion of compound interest (polycyclic) epidemics to describe conditions in which infected plants constitute inoculum sources for additional infections during a single growing season. In contrast, in simple interest epidemics, one plant does not infect another in the same season. The notion can be generalized using the concept of site (Zadoks & Schein, 1979): in a monocyclic epidemic, infected sites do not infect healthy sites. According to Zadoks and Schein (1979) monocyclic epidemics occur when the inoculum is originated from a reservoir, such as soil in the case of soil inhabiting pathogens. They also assume that the amount of inoculum in the soil is constant during the vegetation period.

A misconception has emerged in the literature from the confusion between 'disease' and 'epidemic' (Butt and Royle, 1980): Van der Plank's characterization – monocyclic or not – pertains to epidemics, not to diseases. In other words, a disease may, under some conditions, cause a monocyclic epidemic, whereas under different conditions, an epidemic of the same disease should not be considered monocyclic.

Many studies (Napoleão et al., 2005, McDonald & Boland, 2004, Heffer Link & Johnson, 2007), have addressed epidemics caused by *S. sclerotiorum*, considering them as monocyclic. A few studies have addressed individual types of white mould propagules. In these studies, estimates of sclerotia (Wu et al., 2008, Schwartz & Steadman, 1978), apothecia (Mitchell & Wheeler, 1990, Wu & Subbarao, 2008, Boland & Hall, 1988), ascospores (Clarkson et al., 2003, Caesar & Pearson, 1983, Qandah & del Río Mendoza, 2011), or colonized petals (Turkington & Morrall, 1993, Jamaux et al., 1995) have been examined individually for measuring the efficiency of the initial inoculum of *S. sclerotiorum* and relating these variables to the incidence or severity of

disease. Nevertheless, to the best of our knowledge, no study has yet quantified the set of four different propagule types (and the inoculum sources they constitute) in a experimental systems-based approach of white mould epidemics, as attempted in the present work.

Four types of propagules are considered in white mould: sclerotia (SC); ascospores, that are accounted for by apothecia (APTOT); petals infected by ascospores, accounted by infected flowers with infectious mycelium (IDF); and infectious mycelium in foliage, accounted by the number of lesions on foliage (NL).

When considering an overview of white mould epidemics in *Phaseolus* bean, the following successive hypotheses were made (Figure 7-overview):

There is a relationship between the amount of sclerotia and number of apothecia.

The fraction of ground cover (FGC) may contribute to the increase in the number of apothecia.

There is a relationship between the number of apothecia and the incidence of diseased flowers and petals.

The fraction of ground covered by the crop foliage (FGC) may contribute to the increase in the incidence of diseased flowers (IDF).

There is a relationship between fraction of ground cover (FGC) and number of lesions (NL).

Tu (1989) considered that petal infections can increase rapidly and be responsible for 78 % of the infections observed on the foliage. The one-celled, small, hyaline ascospores of *S. sclerotiorum* are relatively fragile and store little energy, so

viability and infection efficiency is greatly dependent on optimum environmental conditions inside the canopy, which include prolonged moisture duration and diminished solar radiation. Thus, it is assumed that a greater fraction of ground cover (implying denser canopies) positively impacts the survival, viability and inoculum efficiency of ascospores by providing protection from solar UV radiation and extended moisture on the surface of the petals tissues. Our study indicates that there is a close relationship between the incidence of diseased flowers and the number of white mould lesions after flowering (NL). (Figure 7-overview; Figure 2). The fraction of ground cover may also favour the increase of white mould lesions from diseased flowers (Figure 7-overview). Therefore, the number of white mould lesions finally results in an increased number of new sclerotia, which are formed by infectious hyphae. The increase of new sclerotia is an increase of the initial inoculum for the next crop, *i.e.*, it materializes the polyetic increase (Zadoks and Schein, 1979) of the disease over growing seasons. This is very relevant for white mould management, since, according to several authors (Adams & Ayers, 1979), *S. sclerotiorum* sclerotia can survive for more than five years in soil.

Due to the relatively low efficiency of ascospore dispersal over long distances this pathosystem has a close relationship between the amount of initial inoculum and disease incidence. Qandah and Del Rio Mendoza (2012) detected a strong linear association between the concentrations of viable, airborne, *S. sclerotiorum* ascospores and disease incidence in canola fields.

The three groups of white mould epidemics considered in the present study differed sharply in their onset dates, rates and terminal incidences and severities. Group A epidemics were slow to establish, developed at a low rate, and led to a very

low terminal incidence and severity (Figure 4d). This kind of epidemics has been termed as tardive by Gäumann (1950), to describe epidemics that progress slowly, are long drawn out, and their curves do not show a "*paroxysmal peak*". Epidemics in group C, nevertheless, established much earlier and very rapidly and had an initially high rate of increase with a later stabilization (Figure 4f). Epidemics in group B displayed an intermediate behaviour, with an initially low rate of increase until 60 DAS, followed by a strong increase of the rate of foliage infections (Figure 4e).

Epidemics in group A, with the lowest terminal disease incidences (up to 5% of infected plants) (Figure 7), had shapes of disease progress curves that are typically described by the monomolecular model (Table 8). These epidemics do not show a significant increase in the amount of any type of propagule. In monocyclic epidemics the amount of inoculum is constant during the vegetation period (Zadoks & Schein, 1979). The amount of propagule 1 (SC) may be considered constant during the vegetation period in all types of white mould epidemics. The amount of propagule 2 (APTOT) can vary between epidemics but may be assumed constant during the flower infection period. However, a delay of apothecia formation and ascospore liberation can hamper or even prevent the start of an epidemic.

Concerning group A, the following successive hypotheses were made: The low fraction of ground cover (FGC) of group A, by contrast with group C, contributes to the decrease of the number of apothecia (APTOT) by the reduction of the rate of sclerotia germination (Figure 7-Group A). When considering the link between the number of apothecia (APTOT) and incidence of diseased flowers (IDF), a reduction in FGC also contributes to a decrease in the incidence of diseased flowers (IDF) due to a decline in the rate of flower infection by ascospores (Figure 7-Group A; Table 4). This study

indicates that there is a close relationship between incidence of diseased flowers (IDF) and number of white mould lesions after flowering (NL) (Figure 7-Group A; Figure 2). The fraction of ground covered (FGC) also contributes to a reduced number of white mould lesions (NL) from diseased flowers (Figure 7-Group A).

In epidemics of group B, characterized by terminal incidences of infected plants comprised between 20 and 60%, disease progress curves did not conform to the typical monomolecular or logistic model shapes (Table 8). The diagram for group B (Figure 7-Group B, Table 4) displays the following successive hypotheses: The fraction of ground cover (FGC) contributes negatively to the process of sclerotia germination and therefore the number of apothecia is not increased substantially in comparison with group A (only a 1/3 increase in APTOT by numerical difference) (Figure 7-Group B; Table 4). In addition, FGC does not favour the incidence of diseased flowers (IDF) as a function of the number of apothecia (again, only a 1/3 increase over group A). However, FGC does contribute to the increase in the number of lesions on foliage (NL) arising from the diseased flowers, by three times as much as in group A (Table 4, Figure 7-Group B) There is a close relationship between incidence of diseased flowers and number of white mould lesions on foliage (Figure 7-overview). In epidemics of group B, the number of white mould lesions (NL) contribute to a significant increase in the number new sclerotia (Table 4). Nevertheless, the amount of fresh, more viable sclerotia formed in the end of the epidemics in group B will certainly contribute to a higher initial inoculum in the subsequent crop.

Epidemics in group C all reached terminal incidences higher than 60% (Figure 4; Figure 7-Group C) and had sigmoid-shaped progress curves better described by the logistic model (Figure 4f) (Table 8). The diagram of group C (Figure 7-Group C)

illustrates the following successive hypotheses that were made: The amount of sclerotia in group C was not statistically different when compared with the other epidemic groups (P = 0.22) (Table 2). Lack of correlation between initial inoculum and severity of epidemics has been observed equally by growers and researchers (Schwartz & Steadman, 1978). The fraction of ground cover contributes, as a factor, to an increase in the number of apothecia (Figure 7-Group B). FGC also contributes to increases in the incidence of diseased flowers, by increasing ascospore infection efficiency (Table 4; Figure 7- Group C) and the number of lesions in canopy too, by diseased to healthy tissue spread.

Secondary infection is proportional to the amount of infected tissues and also depends on the amount of tissues still available for infection (Savary, 1996, Savary et al., 1997). During the course of epidemics in group A, the plant-to-plant spread (secondary infection) of white mould occurs rarely because of the small frequency of effective contacts. Consequently, the number of lesions (7.27 lesions per microplot) and the incidence of diseased plants (0.11) (Figure 4d) are very small. Boland and Hall (1987) considered that some secondary infections can develop by spread of mycelium from diseased to healthy tissues, but they considered this infection process responsible only for a minor portion of the dynamics of white mould. Heffer Link and Johnson (2007) also considered that plant-to-plant spread of *S. sclerotiorum* may occur occasionally, while considering it a rather rare event. This study strongly suggest that process contagious of plant-to-plant, *i.e.*, "secondary", infection rarely occurs on epidemics of group A.

Plant-to-plant spread of *S. sclerotiorum* can be classified as an "effective contact" phenomenon. According Savary et al. (1997), the operational definition of this process

is a contact between healthy host and infected host tissues that leads to disease. Therefore, for white mould epidemics, the proportion of plants that become diseased depends on the frequency of effective contacts between host and propagules.

The epidemics in group A probably involve few effective contacts because of the reduced fraction of ground cover (reduced plant growth) and consequently decrement numbers of propagule 2, propagule 3 and propagule 4. These observations suggest that the disease is in part "canopy-borne" (Savary et al., 1997). Sclerotia of *S. sclerotiorum* survive in the soil for long periods and, in the initial stage of the disease cycle, white mould can be considered as a soilborne pathogen, in a manner similar to southern blight, caused by *Sclerotium rolfsii* (Rivard et al., 2010, Leoni et al., 2014). After sclerotia germination and apothecia formation, ascospores are released into the air and *S. sclerotiorum* may be regarded as an airborne pathogen. At this stage of disease cycle, white mould can be similar to other diseases, such as apple scab caused by *Venturia inaequalis* (Hirst & Stedman, 1962, Rossi et al., 2007). The reduced fraction of ground cover, leading to reduced contact among vegetative host tissue, decreases the frequency of effective contacts between host and propagules. At this stage, white mould may be regarded as canopy-borne in a manner similar to some other diseases, such as sheath blight (Costa-Coelho et al., 2014). Group A epidemics have a high level of sclerotia per square meter (151 sclerotia.m^{-2}), not significantly different from groups B or C (Table 4). Similar levels of sclerotia inoculum were employed by Huang et al. (2000) and Geraldine et al. (2013) to study bean white mould in the field, and both studies reported high severity levels. On the other hand, group A presents low carpogenic germination of sclerotia (mean value of 9 apothecia.m^{-2}). This suggests that the overall microclimate conditions prevailing inside the canopy of group A field plots

of were not favourable to carpogenic germination. Environmental factors governing sclerotia germination and development of apothecia include soil temperature, soil moisture and solar radiation (Phillips, 1986, Ferraz et al., 1999, Bardin & Huang, 2001). Similar studies were discussed by Boland and Hall (1988). Certainly other factors such as duration of plant surface wetness, air temperature, relative humidity and irradiance play a role in modifying the amount of disease associated with a given level of inoculum.

The optimum temperature-range for apothecia formation is 15-20 °C (Bedi, 1962) and high soil moisture is also required for apothecial development (Abawi & Grogan, 1979). Low FGC in group A epidemics did not ensure the microclimate conditions for apothecia formation (soil temperature, soil moisture) and ascospore infection efficiency. The low amount of apothecia during the bean flowering period contributed to the low incidence of diseased flowers (IDF). Nonetheless, the small fraction of ground cover has probably reduced the amount of effective contacts between infected petals and healthy plants, which led to low incidence of white mould on foliage. White mould epidemics of group A are characterized by low final disease incidences and low density of apothecia (APTOT). This group presented a low amount of infected flowers as well (Table 4).

Group B has moderate intensity epidemics but did not differ statistically from groups A or C with respect to quantities of germinated sclerotia. This group neither differed from group A with respect to the incidence of infected flowers. Nevertheless, group B showed higher final disease incidence than group A. This is probably due to an enhanced efficiency of propagule 3, which resulted in a higher number of lesions (NL,

Table 4). Probably, higher FGC favoured the microclimate conditions for propagule 3 infections.

The final disease incidence in group C is the highest among all groups, with some epidemics reaching up to 100% of the plants infected. The discriminant function analysis revealed that the fraction of ground cover (FGC) was the most important parameter to categorise the epidemic groups (-0.83). These results agree with the published literature, in which the environmental factors influence the sclerotia germination, development of apothecia, petal infection and foliage infection (Phillips, 1986, Bardin & Huang, 2001). The microclimate conditions associated to the different FGCs modify the amount of disease associated with a given level of initial propagule and promote a wide range of white mould epidemics.

Our study reveals a close relationship between incidence of diseased flowers and number of white mould lesions on foliage in group C (Figure 7-Group C). The fraction of ground cover also supports the increase in the number of white mould lesions from diseased flowers (Figure 7-Group C). Number of lesions in the canopy in group C was approximately 11 times higher than in group A. According to Savary et al. (1995) contacts between healthy and diseased plant tissues are efficient avenues for the colonisation of new sites, enabling the spread of the disease. Infected plants with a great number of canopy lesions (NL) will provide a much larger chance of infecting healthy plants, than infected plants with only one infection. In turn, these lesions subsequently favour the increase in the number of new sclerotia. During the course of epidemics in group C, the plant to plant spread (*i.e.* secondary infections) occurs frequently because of the higher frequency of effective contacts. Consequently, the

number of lesions (80.52 lesions per microplot) and the incidence of diseased plants (0.81) (Figure 4d) are also much higher.

Temporal analyses showed that the monomolecular model has a better fit with most white mould epidemics of group A, in agreement with the hypothesis that the initial inoculum (sclerotia, propagule 1) is most relevant for this epidemic group. However, when considering epidemics of higher incidences, as the ones in groups B and C, the logistic model generally showed a better fit. These results confirm the hypothesis that the plant to plant spread (infectious hyphae, propagule 4) of white mould may be most relevant in these latter circumstances. The importance of FGC, SC and APTOT in the disease cycle is reinforced by the discriminant analysis, which classified 63% of the observed epidemics in the correct group, using those three experimental variables as discriminant functions (Table 7).

Epidemics of bean white mould, occurring in similar macro-environmental conditions, with similar amounts of primary inoculum in the form of sclerotia, may follow widely different dynamics. This study indicates that the role of propagules and consequently secondary infection on white mould epidemics may be more relevant than it is currently described and should be taken into account for the understanding of the mechanics of bean white mould. These multi-propagule epidemics are complex, and the variation in epidemic pattern is evident, as illustrated here. The relevance of the propagule type on the dynamics of bean white mould epidemics make it difficult to adequately describe the disease process from the monocyclic and polycyclic patterns standpoint alone ('simple interest' and 'compound interest' sensu Van der Plank 1963).

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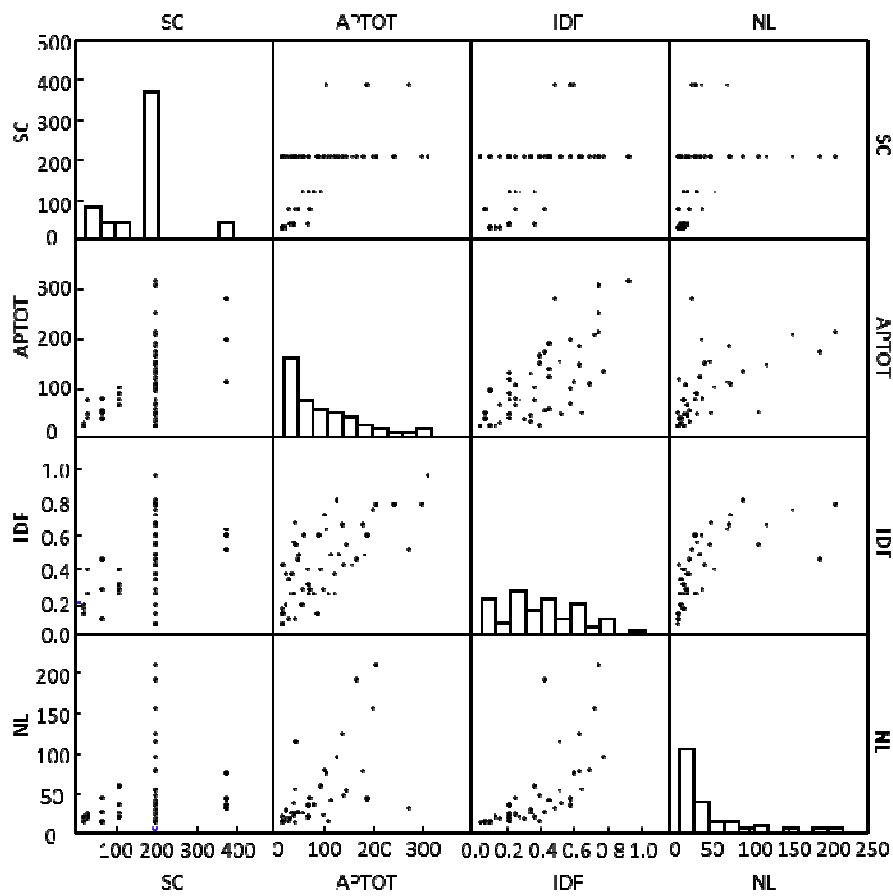
Figure 1. Scatter plots of the relationships between experimental variables. SC: number of sclerotia per microplot. APTOT: amount of apothecia per microplot. IDF: Proportion of infected petals per microplot. NL: amount of white mould lesions per microplot.
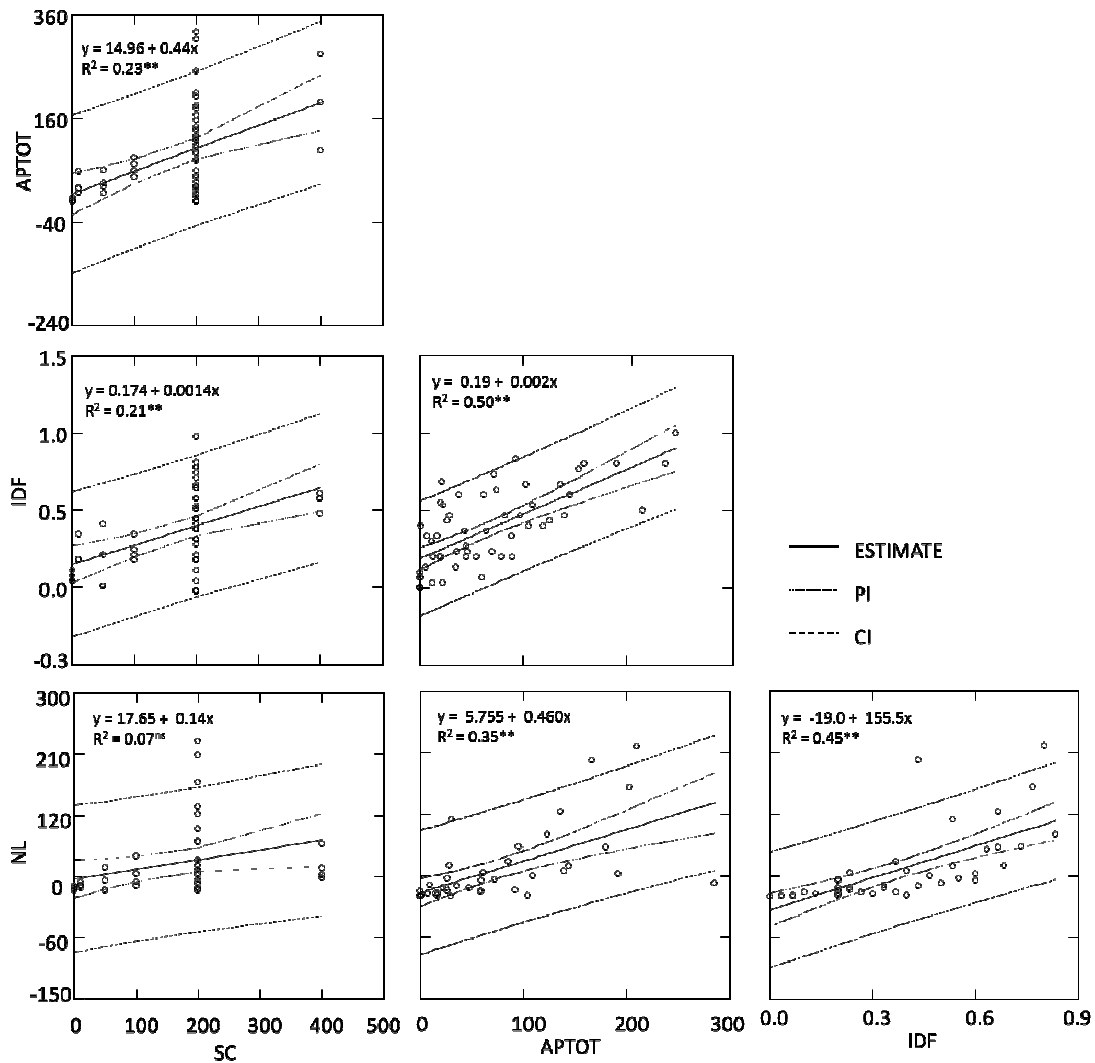
Figure 2. Linear regression models adjusted for experimental variables. SC: number of sclerotia per microplot. APTOT: amount of apothecia per microplot. IDF: Proportion of infected petals per microplot. NL: amount of white mould lesions per microplot. **Significant at $\alpha=0.01$. ns = not significant ($\alpha > 0.01$). CI and PI correspond to confidence intervals and predicted intervals after model adjustment at 95% confidence.
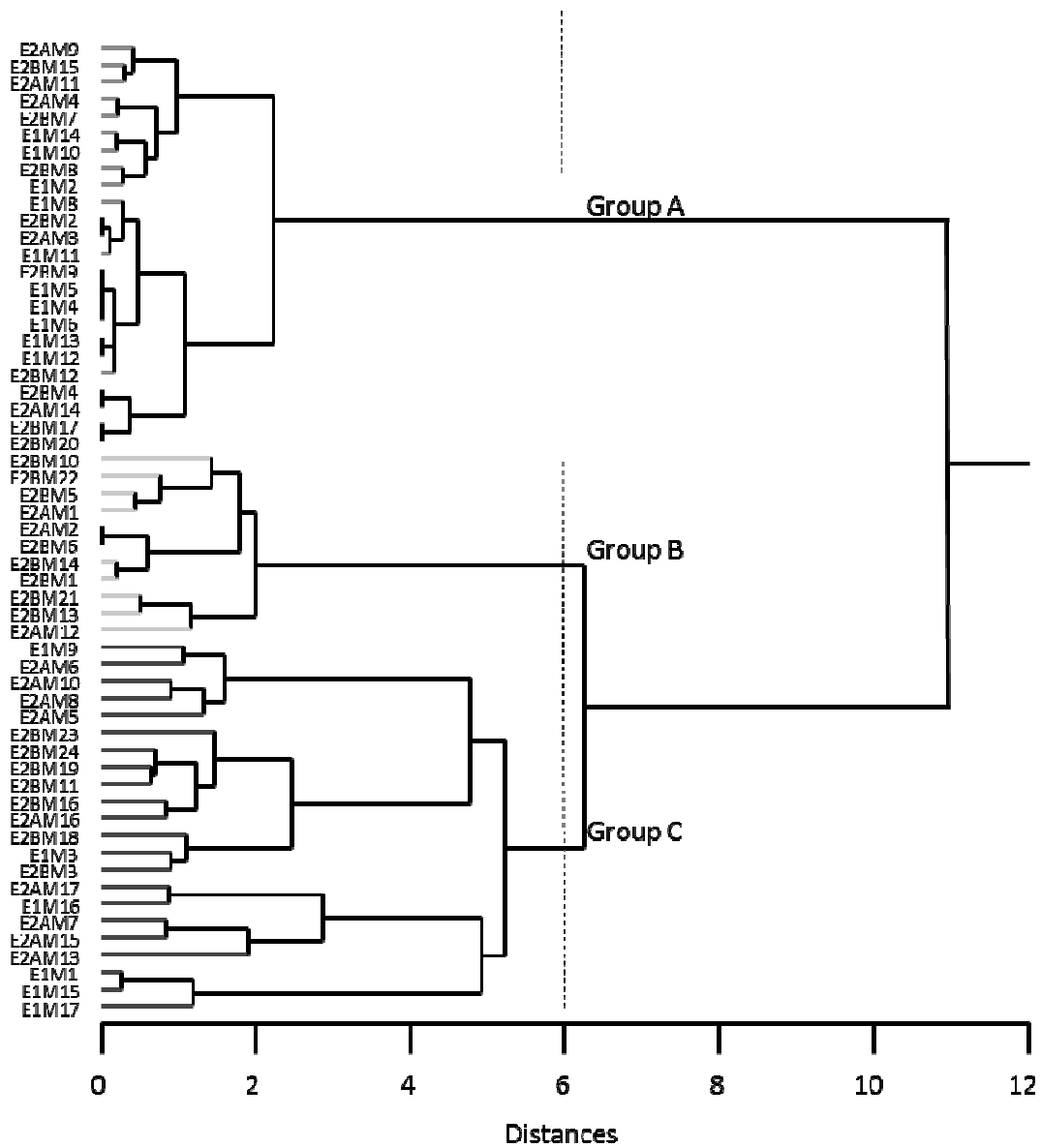
Figure 3. Hierarchical cluster analysis of fifty-seven white mould epidemics by Mahalanobis distance and linkage Ward. Clustering was performed using data from three assessments incidence and disease severity (I_3, I_4, I_5, S_3, S_4, S_5).
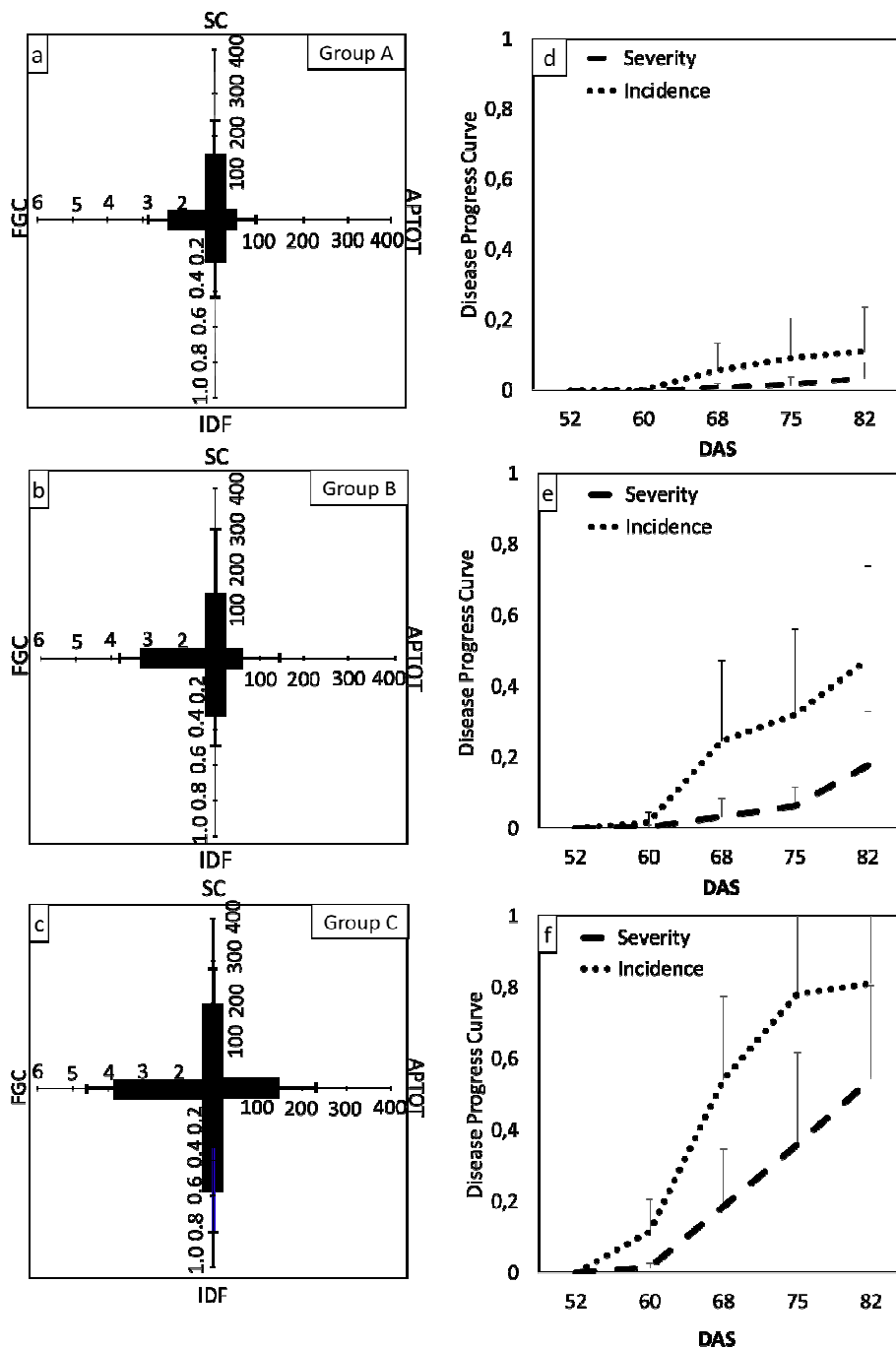
Figure 4. Bar charts of experimental variables and disease progress curves as function of groups generated by hierarchical cluster analysis of fifty-six white mould epidemics. **A**, **B** and **C** represent groups from the cluster analysis. **FGC**: Fraction of ground cover; **SC**: Density of sclerotia; **APTOT**: Density of apothecia; **IDF**: Incidence of diseased flowers; **DAS**: Days after sowing. **a, b, c**: Values of experimental variables for epidemic groups A, B and C, respectively. Disease progress curves summarized by group average. **d, e, f**: averaged progress curves for epidemic groups A, B and C, respectively.
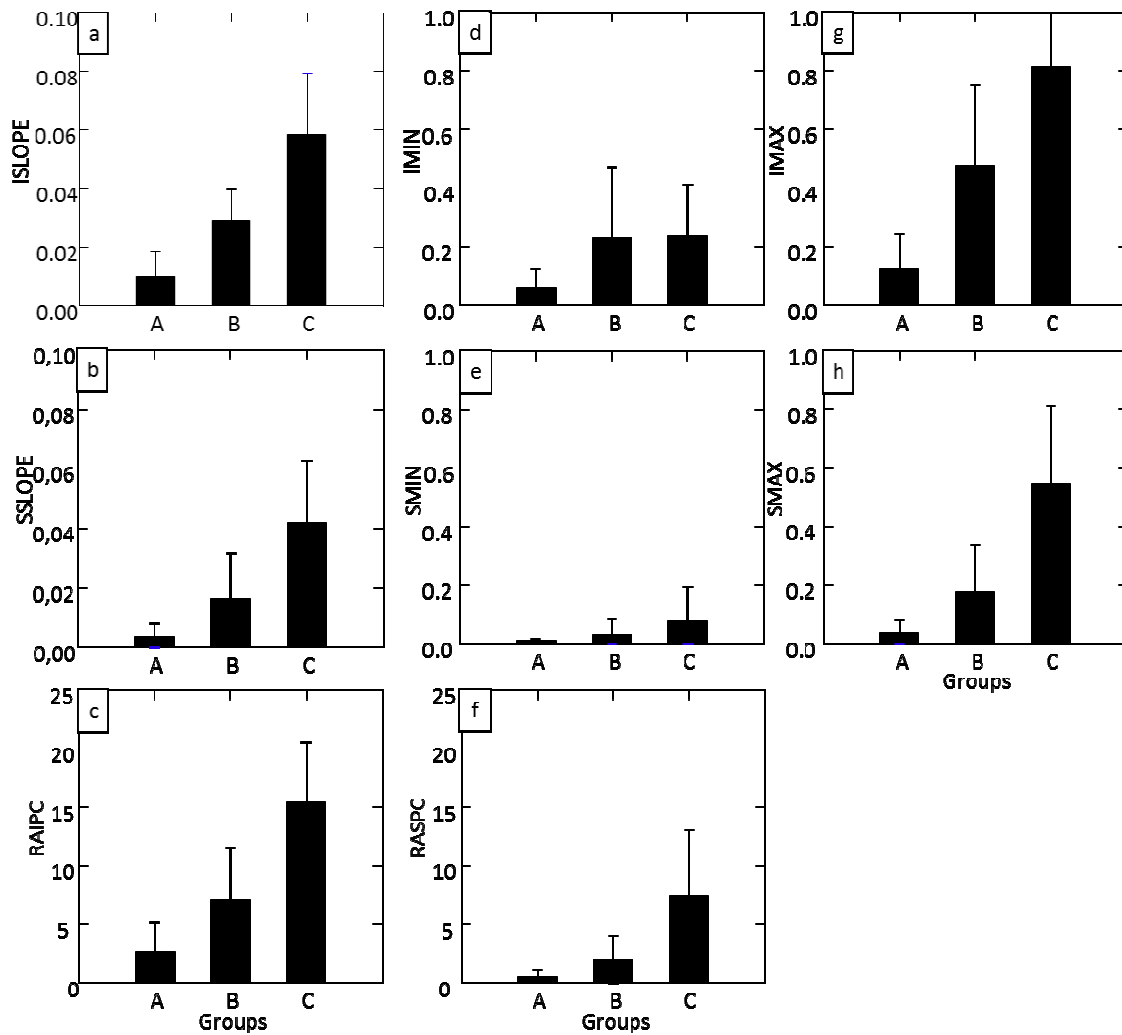
Figure 5. Bar charts of the disease progress curve variables as a function of epidemic groups (A, B and C) generated from hierarchical cluster analysis of fifty-seven white mould epidemics. **a**: Slope of incidence curve; **b**: Slope of severity curve; **c**: Relative area under incidence progress curve; **d**: Minimum incidence; **e**: Minimum severity; **f**: Relative area under severity progress curve; **g**: Maximum incidence; **h**: Maximum severity.
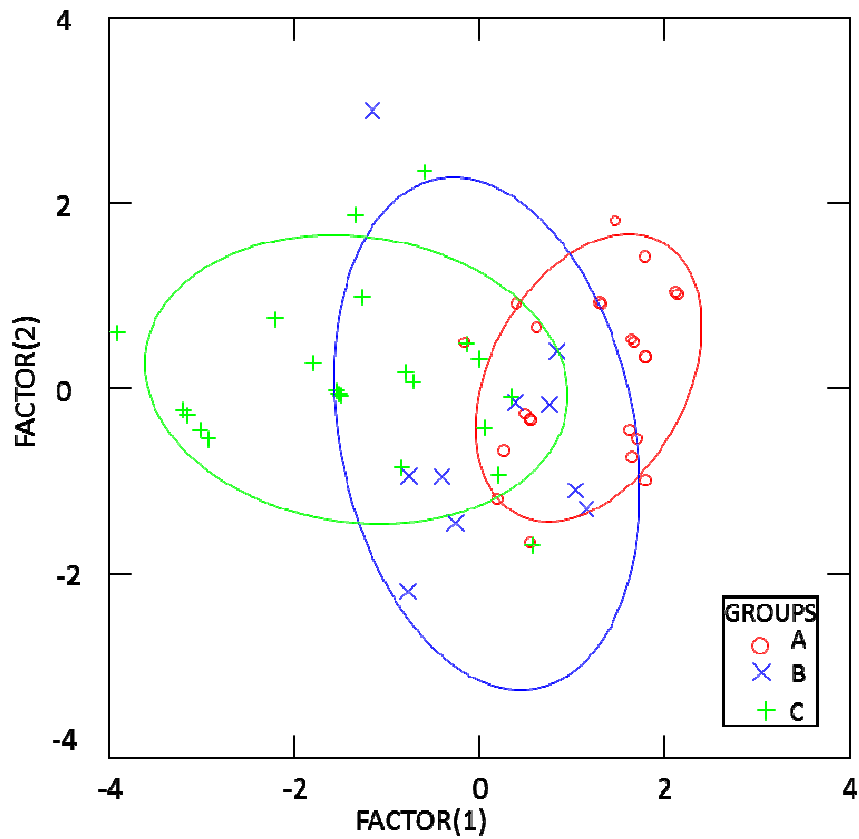
$$F_z = 0.000Z_{SC} - 0.428Z_{APTOT} - 0.833Z_{FGC}$$
$$F_x = 0.609X_{SC} + 0.502X_{APTOT} - 0.458X_{FGC}$$

Figure 6. Discriminant analysis of the experimental variables in function of groups generated from hierarchical cluster analysis of fifty-seven bean white mould epidemics. Discriminant functions were determined as functions of the amount of sclerotia, number of apothecia and fraction of ground cover.
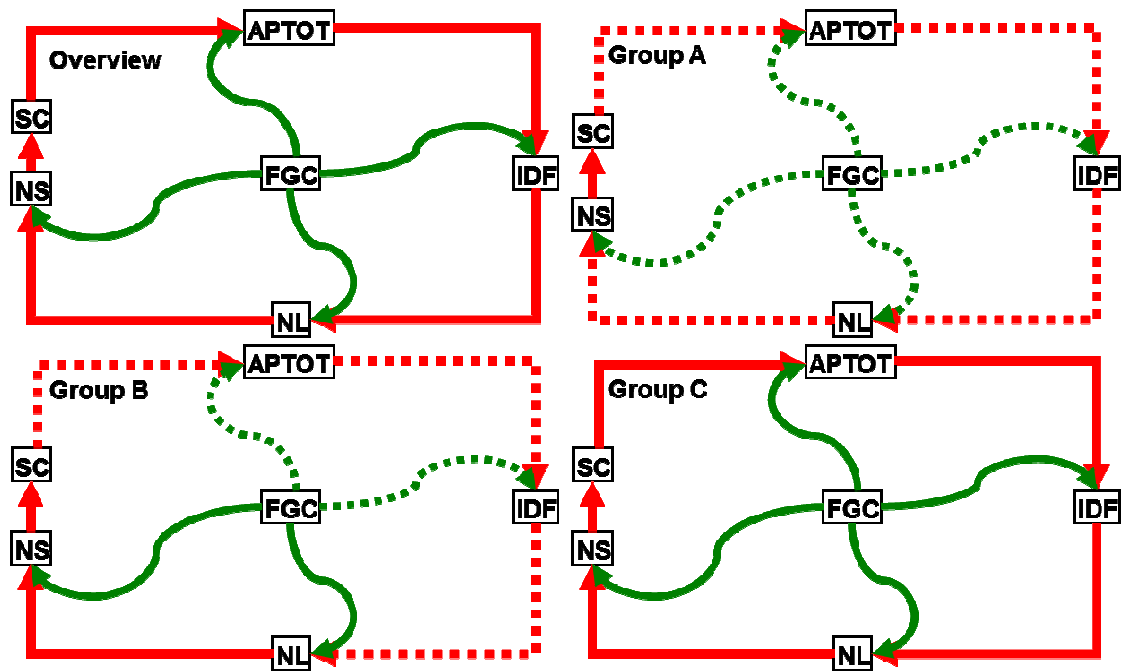
Figure 7. Diagram of white mould cycle and interactions between variables. **FGC**-Fraction of ground cover; **SC**- Initial sclerotium density; **APTOT**- Density of apothecia; **IDF**- Incidence of diseased flowers; **NL**- Number of lesions in the canopy; **NS**- Numbers of new sclerotia. **Overview**: Illustrates the expected interactions in conditions favourable for white mould development. **Group A**: Interactions in group of epidemics with lowest terminal disease incidence (up to 5% infected plants). **Group B:** Interactions in group of epidemics with terminal incidences comprised between 20 and 60% infected plants. **Group C:** Interactions in group of epidemics with disease incidence higher than 60% infected plants. Continuous arrows represent most important flows of infection. Green arrows represents most important relation between FGC and propagules. Red arrows represents most important flows among propagules.

Tables

Table 1. List, symbol, dimensions and units of the experimental and disease progress curve variables

Symbol	Variables	Operational definition	Dimensions	Unit
SC	Sclerotia density	Number of sclerotia on soil surface in a microplot.		
APTOT	Apothecia density	Number of apothecia on soil surface in a microplot.	$[N.L^{-2}]$	Individuals.m^{-2}
I	Incidence	Proportion of diseased plants assessed on 40 plants in two rows on microplot center	$[N.N^{-1}]$	Fraction
S	Severity	Proportion of diseased foliage on two quadrats on microplot center	$[N.N^{-1}]$	Fraction
IDF	Incidence of diseased flower in a microplot	Proportion of diseased flowers in 30 flowers in a microplot	$[N.N^{-1}]$	Fraction
NL	Density of lesions per plant	Number of lesions per plant on 40 plants in two rows on microplot center	$[N]$	Individuals
Y	Yield	Yield estimated at one meter microplot	$[M.L^{-2}]$	kg.ha^{-1}
NS	Density of new sclerotia	Number of new sclerotia in two quadrat on microplot center	$[N]$	Individuals
FGC	Fraction of ground covered	Assessment of ground cover with the 1-5 scale adapted from Deshpande (1992)	[ ]	–
Islope	Slope of incidence curve	Slope maximal of incidence progress curve in a microplot.	$[T^{-1}]$	Day^{-1}
T-Imin	Day of starting disease	Number of days after emergence then diseased was accessed	$[T]$	Day
Imin	Minimum incidence	Minimum incidence above 0 observed in a microplot.	N.N-1=[1]	Fraction
Imax	Maximum incidence	Maximum incidence observed in a microplot.	N.N-1=[1]	Fraction
AUIPC	Area under incidence progress curve	Area under incidence progress curve	$[L^2]$	Diseased plant.day

			Dimension[a]	
RAUIPC	Relative area under incidence progress curve	Area under incidence of progress curve relating to number of days diseased was accessed	[L²]	Diseased plant.day
Sslope	Slope of severity curve	Slope maximum of the disease progress curve in a microplot.	[T-1]	
Smin	Minimum severity	Minimum severity above 0 observed in a microplot.	N.N-1=[1]	Fraction
Smax	Maximum severity	Maximum severity observed in a microplot.	N.N-1=[1]	Fraction
AUSPC	Area under severity progress curve	Area under severity progress curve	[L²]	Diseased plant.day
RAUSPC	Relative area under severity progress curve	Area under severity progress curve relating to number of days diseased was accessed	[L²]	Diseased plant.day

[a]Dimensions: [N]: numbers; [T]: time ; [L]: length.

Table 2. ANOVA table of experimental variables of groups generated from hierarchical cluster analysis of fifty seven white mould epidemics. **FGC**- Fraction of ground covered; **SC**- Density of sclerotia; **APTOT**- Density of apothecia on microplot; **IDF**- Incidence of diseased flowers; **NL**- number of lesions; **NS**- number of new sclerotia

Source	Type III SS	df	Mean Squares	F-Value	p-Value
SC	29,322.8	2	14,661.4	1.550	0.222
Error	510,733.3	54	9,458.0		
APTOT	124,118.4	2	62,059.2	12.593	0.000
Error	261,185.4	53	4,928.03		
IDF	1.3720	2	0.6860	16.158	0.000
Error	2.2920	54	0.0420		
FGC	26.864	2	13.432	30.506	0.000
Error	23.777	54	0.440		
NL	44,690.6	2	22,345.3	12.089	0.000
Error	70,238.6	38	1,848.4		
NS	4,524,183.7	2	2,262,091.8	18.421	0.000
Error	6,631,105.8	54	122,798.3		

Table 3. ANOVA table of disease progress curve variables of groups generated from hierarchical cluster analysis of fifty-seven white mould epidemics. **Islope -** Slope of incidence curve; **Sslope**- Slope of severity curve; **RAIPC**- Relative area under incidence progress curve; **RASPC**- Relative area under severity progress curve **Imin**- Minimum incidence; **Smin**- Minimum severity; **Imax**- Maximum incidence; **Smax**- Maximum severity

Source	Type III SS	df	Mean Squares	F-Value	p-Value
ISLOPE	0.0290	2	0.0150	43.16	0.000
Error	0.0180	54	0.0000		
SSLOPE	0.0170	2	0.0090	40.58	0.000
Error	0.0110	54	0.0000		
RAIPC	1,863.7	2	931.837	45.12	0.000
Error	1,115.2	54	20.6510		
RASPC	582.29	2	291.143	23.80	0.000
Error	660.68	54	12.2350		
IMIN	0.4140	2	0.2070	8.97	0.000
Error	1.2480	54	0.0230		
IMAX	5.4990	2	2.7500	71.64	0.000
Error	2.0730	54	0.0380		
SMIN	0.0570	2	0.0280	5.27	0.008
Error	0.2900	54	0.0050		
SMAX	3.0630	2	1.5320	49.01	0.000
Error	1.6880	54	0.0310		

Table 4. Effect of white mould epidemic groups on experimental variables: **FGC**- Fraction of ground cover; **SC**- Density of sclerotia; **APTOT**- Density of apothecia on microplot; **IDF**- Incidence of diseased flowers; **NL-** number of lesions; **NS-** number of new sclerotia

Epidemic Groups	Arithmetic Mean of Experimental Variables					
	SC	APTOT	IDF	NL	FGC	NS
Group A	150.83 ns	45.38 A	0.24 A	7.27 A	2.29 A	109.79 A
Group B	151.82 ns	60.50 A	0.32 A	22.38 B	3.14 B	364.55 B
Group C	197.73 ns	145.59 B	0.57 B	80.52 C	3.82 C	736.36 C

Means followed by same letters in the column do not differ statistically according to Tukey test (P≤0.05).

ns = not significant (P≤0.05). * = significance level (P≤0.06).

Table 5. Effect of white mould epidemic groups on disease progress curve variables: **Islope -** Slope of incidence curve; **Sslope**- Slope of severity curve; **RAIPC**- Relative area under incidence progress curve; **RASPC**- Relative area under severity progress curve **Imin**- Minimum incidence; **Smin**- Minimum severity; **Imax**- Maximum incidence; **Smax**- Maximum severity.

Epidemic Groups	Arithmetic Mean of Disease Progress Curve Variables							
	Islope	Imin	Imax	RAIPC	Sslope	Smin	Smax	RASPC
Group A	0.01 A	0.06 A	0.12 A	2.71 A	0.003 A	0.01 A	0.04 A	0.55 A
Group B	0.04 B	0.23 B	0.48 B	8.74 B	0.016 B	0.03 AB	0.18 B	2.01 B
Group C	0.06 C	0.24 B	0.81 C	15.45 C	0.042 C	0.08 B	0.55 C	7.48 C

Means followed by same letters in the column do not differ statistically according to Tukey test (P≤0.05).

ns = not significant (P≤0.0 5). * = significance level (P≤0.06).

Table 6. Canonical discriminant functions standardized by within variances. Using fraction ground cover (**FGC**), number of sclerotia per square meter (**SC**) and Number of apothecia per microplot (APTOT)

Factors	Discriminant functions	
	F1	F2
FGC	-0.833	-
SC	0.000	0.609
APTOT	-0.428	- 0.502

Table 7. Jackknifed classification matrix of discriminant analysis performed with 56 bean white mould epidemics using the following experimental variables: **FGC**- Fraction of ground cover; **SC**- Density of sclerotia; **APTOT**- Density of apothecia

	Group A[a]	Group B	Group C	% correct
Group A[a]	16	8	0	67
Group B	4	4	2	40
Group C	0	7	15	68
Total	20	19	17	63

[a]Group A, Group and Group C indicate low, moderate and high intensity epidemic groups, respectively.

Table 8. Summary of linear regressions used in evaluation of three groups for appropriateness for describing disease progress of white mould epidemics

Groups[a]	Total number of epidemics per group	Range of R*² (%)		Number of epidemics with higher R*² values	
		Logistic	Monomolecular	Logistic	Monomolecular
Group A	12	41.8 to 79.7	49.9 to 94.8	2	10
Group B	11	33.9 to 95.7	39.4 to 85.4	7	4
Group C	16	47.2 to 99.8	26.2 to 93.0	9	7

[a] Groups of white mould epidemics identified with hierarchical cluster analysis. **R*²**= Average of coefficients of determination for agreement between observed and predicted incidence.

CHAPTER 3

SIMULATION MODELLING OF BEAN WHITE MOULD EPIDEMICS

SIMULATION MODELLING OF BEAN WHITE MOULD EPIDEMICS

ABSTRACT

Simulation modelling is an efficient approach to conceptualize the structure of a pathosystem and to enable the identification of knowledge gaps. This chapter describes the structure of a model for bean white mould epidemics that emphasizes the successive types of pathogen propagules and their interaction with host plants. We followed five steps in the development of a mechanistic simulation model for white mould on beans: (1) model conceptualization; (2) model verification; (3) parameter estimation; (4) model evaluation; and (5) sensitivity analysis. An ethograph describing the infection cycle in bean white mould was developed and a simplified model was built using the STELLA® 10.6 programme (isee systems, USA). Model verification was performed by running the model and examining the numerical performance and the outputs of each part of the model. Next, eight model parameters were set to vary in four levels for model evaluation. Sensitivity analyses and statistical methods were then conducted in order to identify gaps, which inputs contribute most to output variability and which parameters are most highly correlated with the outputs. Sensitivity analysis of the sclerotia germination sub-model showed that changes in the relative rate of germinated sclerotia (RRGSC) caused variation in the area under disease progress curve (AUDPC) that were smaller than those caused by variation in the parameter initial number of sclerotia (SC). Sensitivity analysis of the flower infection sub-model showed that changes in values of the relative rate of flower senescence (RRFS) caused narrower AUDPC variation than changes in the relative rate of flower infection, RRFI. Different analyses (analyses of variance and principal component analysis) indicate that SC, RRGS,

RRFI, RRP and RRS were the most important parameters of the white mould epidemic simulation model. Three epidemics groups were established using the outputs of the model (AUDPC values) as a criterion for group formation. As a result three epidemic groups were defined by discriminant analysis involving a combination of parameters of the white mould simulation model. Clearly, the bean (*Phaseolus vulgaris*) - *Sclerotinia sclerotiorum* pathosystem is much more complex than the theoretical general "simple interest" epidemics, even if the amount of initial inoculum (in the form of propagule 1, sclerotia) remains as one of the most important parameters, as shown by sensitivity analysis.

1 INTRODUCTION

White mould epidemics are caused by *Sclerotinia sclerotiorum,* a necrotrophic fungal pathogen in the Ascomycota. Although *S. sclerotiorum* is usually treated as a soil-borne plant pathogen in the literature (Smith, 1972), the white mould disease cycle involves aerial dispersal of the pathogen. Many epidemiological sub-processes can be identified, including: sclerotium germination, ascospore liberation, ascospore transport, ascospore deposition, petal infection, foliage infection, and sclerotia production. Many of these processes have been studied separately (Schwartz & Steadman, 1978, Boland & Hall, 1988b, McCartney & Lacey, 1991, Turkington et al., 1991) to varying degrees, but there has not, to our knowledge, been an attempt to model the epidemic process of white mould as a whole, which has generally been considered in the literature (Fry, 1982) as a 'monocyclic' (or 'simple interest') epidemic (Van der Plank, 1963).

Disease spread in the bean (*Phaseolus vulgaris*) - *Sclerotinia sclerotiorum* pathosystem depends on four kinds of propagules. These propagules correspond to the formation and the biological functions of four different types of functional propagules (Butt and Royle 1980): The first propagules are sclerotia, which may germinate by forming saucer-shaped ascocarps called apothecia. The second type of propagules consists of ascospores that are released in the air from apothecia. Bean petals that have been infected by *S. sclerotiorum* ascospores play the role of a third kind of functional propagule which spread disease from flowers onto the foliage of the same or neighbouring plants. The fourth kind of functional propagule consists of infected host vegetative tissues (leaves and stems) where infectious hyphae progress from lesions to new sites through tissue contacts, and cause new infections.

Epidemiology deals with processes, and an epidemic, as a process, consists of sub-process (Zadoks & Schein, 1979). Plant disease epidemics may be considered as inter-connected building blocks, each building block corresponding to an epidemiological process of its own (Zadoks & Schein, 1979, Savary & Willocquet, 2014). Epidemics of white mould can be decomposed into four groups of processes (Figure 1):

- Germination of sclerotia, corresponding to sub-process 1;

- Apothecia formation and ascospore liberation, corresponding to sub-process 2;

- Flower infection (infectious mycelium in petal), corresponding to sub-process 3;

- Foliage infection (infectious mycelium in tissues), corresponding to sub-process 4;

Each of the four infection sub-processes is associated with one kind of propagule. The constituent propagule of the sclerotia germination process is the sclerotium (propagule 1). Following apothecium formation and development of asci, propagule 2 units (ascospores) are formed. In the process of flower infection, propagule 3 entities (infected petals) are produced. Lastly, foliage infected by actively growing hyphae represents propagule 4, which infects healthy host tissues.

The combinations of environmental factors and propagule infection efficiencies rates make epidemics vary from low to high intensities. Changes in the environment and host susceptibility are important in determining the shape of the disease progress curves (Pfender, 1982). For example, soil temperature, soil moisture, and quality of light radiation (Phillips, 1987, Ferraz et al., 1999, Bardin & Huang, 2001) directly affect the process of carpogenic germination. Furthermore, free water is required for ascospore germination and infection (Bardin & Huang, 2001). Under favourable conditions, the quantity of propagules

2, 3 and 4 increases due to a higher relative rate of propagule production. In these situations, the white mould epidemics can be devastating, and cause important yield losses. Conversely, when environmental variables are not conducive to the disease, only low intensity epidemics will develop.

This chapter describes the structure of a model for bean white mould epidemics that emphasizes on the successive types of pathogen propagules and their interaction with host plants.

Simulation modelling is a powerful approach to conceptualize the structure of a pathosystem and to enable the identification of knowledge gaps (Zadoks, 1971, Teng, 1985, Savary & Willocquet, 2014). Mechanistic simulation models that address a given process (e.g., a plant disease epidemic) consist of several inter-linked sub-processes or group of sub-processes (e.g., pathogen dispersal, infection) (Zadoks & Schein, 1979). This approach is useful to identify the relative importance and the numerical contribution of each kind of propagule to the epidemic process of bean white mould and to identify the main parameters that govern epidemics in this pathosystem. Simulation modelling therefore allows to quantify sub-processes that are underpinning epidemics (Savary & Willocquet, 2014) and thus, allows visualization of the behaviour not only of the process, but of the constituent sub-processes.

2. MATERIALS AND METHODS

2.1 Sequential steps taken for the model development

According to Penning de Vries (1982) a simulation model is developed in five steps: (1) model conceptualization; (2) model verification; (3) parameter estimation; (4) model

evaluation; and (5) sensitivity analysis. We followed these steps in the development of a mechanistic simulation model for white mould on beans.

2.1.1 Model conceptualization

As the first step for model development, an ethograph (Savary, 1987) was constructed, summarizing graphically the knowledge pertaining to the infection cycle of bean white mould (Figure 1). According to Savary (2007) drawing an ethographs implements basic biological knowledge; for botanical epidemiologists it however serves the important purpose of locating where environmental factors may play important roles in epidemics. In order to develop the structure of a mechanistic model for the disease, we used a simplified version of an ethograph of white mould of bean (Figure 1).

As indicated above, four types of propagules were considered: (1) sclerotia, (2) ascospores, (3) infected petals, and (4) infectious hyphae in vegetative host tissue in the canopy. We then converted the simplified version of the ethograph into the flowchart of a mechanistic simulation model encompassing the four sub-processes pertaining to these different propagules. The overall structure of white mould simulation model is shown in Figure 2.

The structure of the model was developed using symbols and concepts developed by Forrester (1961) for system analysis: state variables, rates, parameters, flows of materials or individuals, flows of information, and driving functions. Forrester symbols and syntax employed in this work are as follows:

State variable that can be measured.

Flow of materials or individuals from one state variable to another.

─ → Flow of information (between state variables and rates, coefficients and state variables, coefficients and rates, and between coefficients).

◯ Coefficients (fixed or calculated).

⬚ Rate of transfer from one state variable to another.

The structure of the model was built using the STELLA® 10.6 program (isee systems, USA). This software enables to focus on the components of a system and on the relationships between components and parameters, rather than on the codes of the program itself (Savary & Willocquet, 2014).

2.1.2 Model verification

Model verification consists in verifying that the model actually performs the calculations as programmed (Zadoks, 1971, Penning de Vries, 1982). Model verification was performed by running the model and examining the numerical performance and the outputs of each part of the model. The procedure consists in verifying the overall behaviour of the model, and observing if the outputs conform with patterns of disease progress described in a number of independent reports (Savary & Willocquet, 2014).

2.1.3 Variation of parameter values

Parameters were varied, each one in turn, within ranges derived from the literature. Each of the parameters was set to vary in four levels. These parameter values were chosen using logical criteria accounting for values that can be observed in actual field conditions. Values for relative rates were also determined in accordance with levels observed in field conditions.

Additional parameters were included in the model using information retrieved from the experimental field epidemics studied in Chapter 2. For example, the quantity of sclerotia

in the system was the same used in the field experiments of Chapter 2. Parameters values are discussed in sensitivity analysis of the model.

2.1.4 Model evaluation

The objective of model evaluation is to analyse the outputs generated by the model so that potential flaws are detected. These flaws include improper association among the chosen variables or their respective dimensions. Each of the four components of the model (see below) was individually evaluated using its specific outputs, in order to forward judgement on the performance of the model.

2.1.5 Sensitivity analysis

Sensitivity analyses were conducted in order to determine: (1) which parameters require additional research on the white mould epidemic process; (2) which inputs contribute most to output variability, i.e., variability of epidemics; and (3) which parameters are most highly correlated with the outputs (Hamby, 1994).

There are many different ways of conducting sensitivity analysis. Here, we used the simplest method which consists of quantifying the changes in outputs in response to the changes effected in input values of one parameter, while all other parameters remained constant (Krieger et al., 1978). The successive sensitivity analyses were done using the area under disease progress curves (AUDPC; Shaner & Finney, 1977; Campbell & Madden, 1990) as the output value in order to identify which parameter contributes most to output variability.

2.2 Development of a simulation model structure for white mould epidemics

The ethograph describing the infection cycle of bean white mould (Figure 1) was converted into the flowchart of a simulation model encompassing four sub-processes corresponding to each respective pathogen propagule. (Figure 2a).

A propagule was defined as a pathogen entity that is formed, dispersed, and may cause new infections (Van der Plank, 1963). Agrios (2005) defines a propagule as the part of an organism, such as a spore of a pathogen, that may be disseminated and reproduce its thallus. We considered four types of functional propagules for this simulation model: propagule 1 = sclerotia; propagule 2 = ascospores; propagule 3 = infected petals (infectious hyphae in petals); propagule 4 = infectious hyphae in the host foliage.

Each sub-process in this model represents a component of the life cycle of the pathogen and was specifically related to one of these types of propagules. The resulting model structure (Figure 2a) is fairly complex, and involves a rather large number of parameters. Figure 2a shows in green those processes of the disease cycle which were not directly quantified in the present study. Using the framework of Figure 2a, a simpler structure was designed (Figure 2b), based on (i) the four types of propagules considered in this study, and (ii) those stages of the disease cycle which were actually measured in this study (processes A , D, F, G, Figure 2b).

Figure 2b shows a model structure that includes the most important sub-processes of the infection cycle. Each sub-process represents the life cycle of the pathogen related to one propagule. This approach takes into account three of the four types of propagules identified on the bean white mould pathosystem: propagule 1 (sclerotia); propagule 3 (infected petals as infectious hyphae in petal); propagule 4 (infectious hyphae in the host foliage). The processes pertaining to propagule 2 (ascospores), i.e., apothecia and ascospore formation,

ascospore liberation, and ascospore deposition, were not specifically addressed and are instead embedded as part of other sub-models processes.

Three sub-models are shown in the model structure of Figure 2b. The first one (sub-model 1) concerns the process of sclerotia germination (propagule 1). The second sub-model (sub-model 2) refers to the process of bean flower infection (propagule 3). Lastly, sub-model 3 is related to the process of bean foliage infection (propagule 4).

Sub-model 1 quantifies the processes of sclerotia germination and of sclerotia removal from the system. Sclerotia can be considered germinated when stipes which will bear apothecia starts developing from the medullary hyphae beneath the rind of the germinating sclerotia (Saharan & Mehta, 2008, Coley-Smith & Cooke, 1971). For the purpose of this model, germinated sclerotia are carpogenically germinated-sclerotia, that is, sclerotia with apothecia. Therefore, removed sclerotia are sclerotia that, after a period of one to two weeks (Clarkson et al., 2003) are no longer able to develop new apothecia.

Figure 2b also shows the replacement of some sub-processes by their respective relative rates of infection (red circles). The ascospore formation process is represented in a condensed way by the relative rate of flower infection as a function of germinated sclerotia (RRFI).

The second part of the model describes the sub-processes of flower infection, flower senescence and rate of flower removal (Figure 2b). Sub-model 2 corresponds to propagule 3 of the ethograph (Figure 1), infected flowers which petals had been infected by ascospores and were colonized by *S. sclerotiorum* mycelium. The number of healthy flowers was fixed (1500 flowers.system area^{-1}) since the quantity of flowers per plant is an intrinsic characteristic for each bean cultivar (Hoffmann Júnior et al., 2007). Thus, we considered

that the number of flowers in the system is fixed. The state variable representing removed flowers refers to infected flowers that have dropped onto the ground, and which are no longer able of causing new infections. The flower infection sub-process is divided into individual petal infections that constitute the smallest unit of infection. This sub-process was considered in a simplified way by the relative rate of primary infection on foliage (RRP) where flowers (propagule 3) were considered the smallest infection units (Figure 2b).

The third part of the model summarizes the sub-processes that describe the dynamics of primary infection on foliage and of the secondary infections by spread of mycelium (infectious hyphae on host tissue) from diseased tissues to the healthy ones (Figure 2b). Firstly, primary infection on foliage occurs when infected flowers (propagule 3) make contact with healthy foliage tissues and cause new infections. Secondary foliage infection can then develop by spread of mycelium from diseased tissues (infectious hyphae, propagule 4) to healthy ones. This infection process originating from the contact of diseased tissues with health tissues was represented in a simplified manner by the relative rate of secondary infection (RRSI) (Figure 2b).

2.3 Overall structure of the model

Model construction was based on a number of hypotheses, with a focus on the validity of the model structure. A first hypothesis concerns the surface area of model system. Actual white mould epidemics were simulated in this model by a virtual bean crop unit sized 5 m^2, represented by a fixed population of 50 plants, surrounded by identical systems. This system size corresponds to the size of the experimental units studied in Chapter 2, where observations were made. The implicit hypothesis of the 5 m^2 unit surrounded by identical systems is that the amount of disease is the same in surrounding areas and also infers that

the crop structure does not vary greatly. All these assumptions held true during the field experiments carried out in Chapter 2. Zadoks (1971) and Savary et al. (2012) considered that in wheat and rice, respectively, epidemics caused by a wide range of diseases can be addressed considering simulation units consisting of a 1 m^2 crop area. Because a bean crop canopy may not be as homogeneous as that of a cereal crop, we considered a larger area (5 m²), in order to seek homogeneity of the system in terms of crop structure and amount of disease. A further assumption in the model is that we used a one-day time step and the simulation started 35 days after bean sowing date. Start of modelling was chosen in function of the start of the germination of sclerotia of *S. sclerotiorum* in the bean field that correspond to conditions when canopy closes. Observations in experimental plots showed that sclerotia germination started approximately 35 days after bean planting date, coinciding with the beginning of bean flowering. Other hypotheses will be discussed in each respective model sub-processes, as necessary.

Modelling propagule 1: Sclerotia

Germination of sclerotia (propagule 1) is the first sub-process of the model. Sclerotia may remain viable in the soil for seven years or more (Coley-Smith & Cooke, 1971). Although a sclerotium may also germinate by means of mycelium eruption through the sclerotium wall, this is generally considered negligible in quantitative terms, and ascospores have the predominant role as the primary infection source (Newton & Sequeira, 1972), and specifically for bean white mould (Abawi & Grogan, 1979).

In the sclerotia germination sub-model, the first assumption is that the sclerotium is the smallest unit of propagation. We also consider that any sclerotium may belong to only one of the three (non-overlapping) categories: not germinated (SC); germinated (GSC); or

dead (DSC) (Figure 3). The list of state variables, rates and parameters described on bean white mould model is given in Table 1. The number of sclerotia in the system was written as:

$$SC(t) = SC(t - dt) + (-RGS) * dt,$$

where RGS is the rate of germinated sclerotia (Figure 3).

The number of germinated sclerotia can be calculated as:

$$GSC(t) = GSC(t - dt) + (RGS - RDS) * dt,$$

where RDS is the rate of death of sclerotia (Figure 3).

Modelling propagule 2: Ascospores

The sub-processes of apothecia formation and ascospore liberation give rise to the second type of propagules (propagule 2), i.e., ascospores (Figure 2aB). Propagule 2 is the most numerous type of propagules in the considered system: each apothecium can produce up to 2×10^6 ascospores released continuously during the entire life of an apothecium, which on average lasts 9 days (Schwartz & Steadman, 1978). Due to the lack of information about the production of ascospores and their infection efficiency on bean petals, ascospore production was collapsed in the broader process of petal infection shown in Figure 2b.

Modelling propagule 3: Infected petals

Infected flowers can be divided into individual infected petals that constitute the smallest units of infection in this sub-process. However, for model simplification, infected flowers were considered the smallest units of infection. Here we also supposed that flowers may belong to one of the two (non-overlapping) categories: healthy (HF) or diseased flowers (DF). However, it is important to note that even when petal infection occurs, the disease

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process may not proceed. For the completion of plant infection the infected petals have to fall onto other organs, where infection of vegetative tissue may occur.

The number of healthy flowers (HF) in function of time can be calculated as:

$$HF(t) = HF(t - dt) + (RFI - RFS) * dt,$$

where RFI is the rate of flower infection and RFS is the rate of flower senescence.

Infected flowers (diseased flowers) had had their petals infected by ascospores. The number of diseased flowers was calculated as:

$$DF(t) = DF(t - dt) + (RFI - RRF) * dt,$$

where RFI is the rate of flower infections and RRF is the rate of removed flowers.

Diseased flowers (DF) are removed from the system by a rate of removed flowers (RRF) that is governed by the relative rate of removed flowers (RRRF). Therefore, removed flowers represent infected flowers that have dropped onto the soil and are no longer able of causing new infections (Figure 3).

Healthy flowers (HF) can also be removed from the system after dropping on the ground through natural decay (flower senescence). The relative rate of flower senescence (RRFS) governs the rate of flower senescence (RFS). Senesced flowers are no longer able of causing new infections. Senesced flowers are calculated as:

$$SF(t) = SF(t - dt) + (RFS) * dt$$

A correction factor (Zadoks, 1971, Van der Plank, 1963), CORF, representing availability of healthy flowers to infection was calculated as:

$$CORF = HF/ (HF + DF + RF + SF)$$

Modelling propagule 4: Infectious hyphae

Bean plants in the system may belong to one of two categories: healthy (HP) or diseased (DP). With respect to foliage infection, two pathways are considered. Primary infection on foliage is generated by infected petals. Secondary infection on foliage may develop from spread of mycelium (infectious hyphae) from diseased tissues to the healthy ones (propagule 4). A similar structure was described by Savary et al. (1997) for sheath blight of rice, caused by *Rhizoctonia solani*, and by (Gilligan, 1990) and Savary (2014) for epidemics with dual sources of inoculum.

Primary infections on foliage result from the dispersal of infected petals, which are dropped and lodge in branch axils or adhere to aerial plant parts, such as leaves, petioles, stems, and developing pods. Contact infection may also take place within the canopy, and results from contacts between healthy and diseased plants or plant parts. The frequency of this type of infection has not been quantified in the literature. Healthy bean plants may become diseased through any one of those four types of propagules, but the great majority of infections are determined by propagules 3 (Tu, 1989a) and 4 (Figure 1).

Thus, the number of healthy plants (HP) is calculated as:

$$HP(t) = HP(t - dt) - (RP + RS) * dt,$$

where RP is the rate of primary infection on foliage and RS is a rate of secondary infection on foliage.

Thus, diseased plants (DP) are calculated as:

$$DP(t) = DP(t - dt) + (RP + RS) * dt$$

The rate of change of the number of infected plants can thus be represented by the sum of the rates of primary and secondary infections (Gilligan, 1990, Savary et al., 1997).

$$dDP/dt = r_p HP(1 - (DP/(HP + DP)) + r_s DP(1 - (DP/(HP + DP)$$

Another correction factor (Zadoks, 1971), CORP representing plant availability was calculated as:

$$CORP = HF/ (HF+DF+RF+SF)$$

Here, CORP accounts for the availability of healthy plants to infection (Van der Plank, 1963, Zadoks, 1971, Savary & Willocquet, 2014).

Petal infection may be written as a rate of primary (index p) infection over time t: $(dDP/dt)_p$

Mycelial contact infection may be written as a rate of secondary (index s) infection over time t: $(dDP/dt)_s$;

These two rates are assumed to be independent, so that an overall rate of disease increase may be written as:

$$dDP/dt = (dDP/dt)_p + (dDP/dt)_S$$

The rates of petal (primary) infection and mycelial contact (secondary) infection may be described by a monomolecular model and a logistic model, respectively (Savary et al., 1997):

$$dDP/dt = r_p \, HP(\, 1 - (DP/(HP + DP)) + r_s, DP(\, 1 - (DP/(HP + DP),$$

where r_p and r_s, are intrinsic rates of petal infection and of mycelial contact infection, respectively (Savary et al., 1997).

2.4 Additional components of the model

Disease incidence was computed as the percentage of diseased plants over total plants:

$$Incidence = (DP/(HP+DP))/100$$

The area under disease progress curve (AUDPC) was calculated using the rectangular integration (Campbell & Madden, 1990a). Incidence of diseased plants was the disease variable for the construction of AUDPCs.

$$AUDPC(t) = AUDPC(t - dt) + (RAUI) * dt$$

Whereas RAUI is the rate of increase of the area under disease progress curve. This equation represents the daily accumulation of diseased plants.

2.5 Analyses on the simulated white mould epidemics

Firstly, simulation outputs come from the model were examined in the model verification step in order to detect structural errors. Simulation outputs were agreeable with the patterns of disease progress described in several independent reports (Aghajai et al., 2010, Clarkson et al., 2014).

Sensitivity analysis was performed considering eight parameters of the model: SC, RRGS, RRDS, RRFI, RRFS, RRRF, RRP and RRS (Table 2). The analysis was carried out within each sub-model using four model outputs: germinated sclerotia (GSC); diseased flowers (DF), diseased plants (DP); area under disease progress curves (AUDPC).

The ability of the model to account for the range of disease incidence that is experimentally observed (Chapter 2) was then assessed. Epidemics groups were thus established using the output of the model (AUDPC values) as a criterion for group formation, with four levels of AUDPC set according to the results of Chapter 2. Each of the four epidemic groups was then characterized by a number of attributes: arithmetic mean, mode, minimum, and maximum value of model parameters SC, RRGS, RRDS, RRFI, RRFS, RRRF, RRP and RRS (Table 2). Statistical comparisons were then performed to assess which of these attributes discriminates the four epidemic groups.

Temporal analysis was carried out according to the methodology described by Campbell and Madden (1990b), pages 172 and 182. Equations with linear parameters from each of two models, i.e. monomolecular (ln $[1/(1- y)]$ = ln $[1/(1-yo)]+r_M t$) and logistic (ln $[y/(1- y)]$= ln $[yo/(1-yo)]+r_L t$), were used as prediction equations to compare linearly transformed data and thus the adjusted coefficient of determination ($R^{2}*$; Campbell and Madden, 1990b) was estimated for each simulated epidemics. Adjusted coefficients of determinations ($R^{2}*$) were estimated for each growth curves model (monomolecular and logistic) in each simulated white mould epidemics.

Principal component analyses (PCA) was performed with all model parameters as variables: SC, RRGS, RRDS, RRFI, RRFS, RRRF, RRP and RRS (Table 2) and also the following model outputs: AUDPC, final disease incidence, and the coefficients of determination of the temporal analyses of monomolecular (MONO) and logistic models (LOGI) as well. This analysis aimed to assess relationships between parameters and to identify which parameters contribute most importantly to the development of the epidemics. PCA also helped identify which parameters contributed to the formation the different intensity epidemic groups.

A discriminant analysis was carried out in addition to PCA to further determine which parameters contribute most to distinguishing epidemic groups. Among the statistical and graphical outputs, discriminant functions were especially examined.

Lastly, analyses of variance were performed on the model parameters according to the groups of epidemics. These analyses aimed to detect possible differences in the mean values for each parameter among groups of white mould epidemics. When significant

effects on epidemic clusters were detected, the Tukey test was performed at the 0.05 level of significance.

3. RESULTS

3.1 Sensitivity analysis of the model

Sensitivity analysis was carried out within each sub-model using the following model outputs: germinated sclerotia (GSC); diseased flowers (DF), diseased plants (DP); and area under incidence progress curves (AUDPC).

In this analysis, eight parameters were chosen for a sensitivity analysis of the model: number of sclerotia in the system (SC); relative rate of germinated sclerotia (RRGSC); relative rate of removed sclerotia (RRDS); relative rate of flower infection (RRFI); relative rate of flower senescence (RRFS); relative rate of flower removal (RRRF); relative rate of primary infection on foliage (RRP); relative rate of secondary infection on foliage (RRS). The eight parameters were set to vary within four levels (Table 2).

The effects of parameter changes were evaluated for each parameter individually. Since the number of parameters is rather large, we decided to conduct the sensitivity analysis in three stages: sclerotia germination; infected flowers; and foliage infection. The successive sensitivity analyses were carried out using the AUDPC as the output value (Table 3).

The first sensitivity analysis was conducted within the sclerotia germination sub-model. The parameters sclerotia (SC), relative rate of germinated sclerotia (RRGS) and relative rate of death of sclerotia (RRDS) were set to vary with four levels (Table 2), while the others parameters were fixed at the following standard values: RRFI=0.10; RRFS=0.05;

RRRF= 0.10; RRP=0.02; RRS=0.05 (Table 2). Sixty-four simulations were therefore performed (Figure 4).

Considering the sclerotia germination sub-model, AUDPC values of simulated epidemics ranged from 16 to 2239 diseased plant • day. When considering changes in initial sclerotia number per microplot (SC), AUDPC average values for the simulated epidemics varied widely, between 95 and 1887 diseased plant • day. Changes in the relative rate of germinated sclerotia (RRGSC) value caused smaller variation of AUDPC compared to the effect of SC. Nevertheless, variations in RRGSC caused AUDPC variations that ranged from 513 to 1329 diseased plant • day (Table 3). Variation of the relative rate of removed sclerotia translated into still narrower AUDPC variability, with average values of AUDPC between 914 and 1158 diseased plant • day.

Additional sixty-four simulations were then performed within the flower infection sub-model (Table 2). Here, the parameters relative rate of flower infection (RRFI), relative rate of removed flowers (RRRF) and relative rate of flower senescence (RRFS) were set to vary, with four levels (Figure 5). The five other parameters were fixed (Table 2), and AUDPCs were used as a reference output value, as in the previous sub-model.

Sensitivity analysis of the flower infection sub-model led to AUDPC values ranging between 122 and 2403 diseased plant • day. Changes in the relative rate of flower infection rate (RRFI) had the stongest effect on AUDPC, which ranged from 427 to 1820 diseased plant • day. Changes in the relative rate of flower senescence (RRFS) caused narrower AUDPC variation than changes in RRFI: the average values for the simulated AUDPC epidemics varied between 795 and 1633 diseased plant • day. By contrast, the relative rate

of flower removal (RRRF) led to the smallest range of AUDPC, which only varied between 1047 and 1493 diseased plant • day.

Lastly, the foliage infection sub-model was evaluated in a third phase of the sensitivity analysis. Here, the parameters relative rate of primary infection (RRP) and relative rate of secondary infection (RRS) were also successively set to four levels, while the six others parameters were set to their standard (default) values. Sixteen simulations were thus carried out (Figure 6). In this third phase of sensitivity analysis, RRP had the strongest effect on AUDPC, which ranged from 1282 to 2426 diseased plant • day . Conversely, RRS lead to a smaller variation between the smallest and largest values of simulated AUDPC, which ranged from 1605 to 2100 diseased plant • day (Table 3).

3.2 Relationship between model parameters by principal component analysis

Principal component analysis was carried out on the 144 simulated white mould epidemics that were simulated in the three phases of the sensitivity analysis described above, each epidemic being represented by 12 variables (Figure 7; Figure 8). All model parameters were used as variables: Number of sclerotia in the system (SC); relative rate of germinated sclerotia (RRGSC); relative rate of removed sclerotia (RRDS); relative rate of flower infection (RRFI); relative rate of flower senescence (RRFS); relative rate of flower removal (RRRF); relative rate of primary infection on foliage (RRP); relative rate of secondary infection on foliage (RRS). The simulated final disease incidence and AUDPCs were also included in the principal component analysis. Coefficients of determination of temporal analysis of monomolecular(MONO) and logistic model (LOGI) from simulated white mould epidemics were used in the principal component analysis as well.

The first axis generated by the PCA accounted for 30.5 % of total variance. This first axis was significantly characterised by the following group of variables ($P < 0.01$): AUDPC; Incidence; SC; MONO; RRFI; RRGSC; RRFS; LOGI. These variables can be divided into two groups, one group including variables with positive correlations with the first factor (SC, RRGSC, RRFI, Incidence, MONO and AUDPC) and the other group with negative correlations with this factor (RRFS and LOGI) (Table 4).

The second axis generated by the principal component analysis accounted for 15.97 % of total variance. A distinct group of variables characterized the second axis ($P < 0.01$): MONO; RRRF; RRFS; LOGI; RRFI; Incidence; AUDPC; RRS (Table 4). These variables can also be divided into two groups, with positive or negative correlations with the second factor of the analysis, RRRF, RRFS, and MONO in the former, RRS, RRFI, Incidence, AUDPC, and LOGI in the latter (Table 4).

3.3 Characterization of simulated epidemic groups

AUDPC values were used as a criterion to partition simulated white mould epidemics in three groups. AUDPC alone does not account for all characteristics of epidemic shapes over time (Campbell and Madden, 1990a). The shape of disease progress curves in each AUPDC-based group was therefore examined separately.

Here, the AUDPC values were quantified by the rectangular method, (Euler integration, Campbell & Madden, 1990). The first group named the Low Intensity Group (LIG) is characterized by white mould epidemics with final incidence levels varying from 0 to 0.21. LIG is characterized by AUDPC less than 731 diseased plant.day values. The second group called Moderate Intensity Group (MIG) is characterized by white mould epidemics with final incidence levels varying between 0.22 and 0.66. Here, MIG is composed by

epidemics with AUDPC value between 731 and 2193 diseased plant.day values. The third group called High Intensity Group (HIG) is characterized by white mould epidemics incidence above 0.66. HIG is characterized by epidemics with AUDPC greater than 2193 diseased plant.day.

Analysis of variance of model parameters among epidemic intensity groups

Analysis of variance (ANOVA) shows significant differences among the three epidemic groups with respect to SC (number of sclerotia on soil per 5 m^2 of bean crop) ($P < 0.01$) (Table 6). ANOVA also indicates that the three clusters corresponded to significant differences in RRGSC (relative rate of germinated sclerotia) ($P < 0.01$), RRFI (relative rate of flower infection) ($P < 0.01$), RRRF (relative rate of removed flowers) ($P < 0.01$) and RRP (relative rate of primary infection on foliage) ($P < 0.01$) (Table 6). However, no significant differences among the three clusters are identified for RRFS (relative rate of flower senescence) ($P = 0.09$) nor for RRDS (relative rate of removed sclerotia) ($P = 0.09$). The effect of the relative rate of secondary infection on foliage (RRS) is also accepted as a significant ($P = 0.06$) factor for the grouping of the epidemics (Table 6).

The low intensity epidemic group (LIG) corresponds to epidemics with AUDPCs lower than 731 diseased plant.day. This group is composed by forty-four white mould epidemics (Table 5). With respect to the number of sclerotia per microplot (SC), group LIG has a mean SC value of 249 sclerotia in the system (Table 7).

Disease progress curves in the LIG group increase according to a simple linear shape after the full bloom stage (Figure 10). This group also has the lowest RRGS (mean relative rate of germinated sclerotia per microplot) value, with 0.076 sclerotia • sclerotia^{-1} • day^{-1} (Table 7). On the other hand, when considering RRDS (mean relative rate of death of

sclerotia), group LIG is characterized by an average RRDS value of 0.12, which is not significantly different from the other groups (Table 7). Nevertheless, with respect to the relative rate of flower infection (RRFI), epidemics in the LIG group have the lowest RRFI value (Arithmetic Mean: 0.066 flower • flower^{-1} • day^{-1}) (Table 5). This implies that 6.6% of healthy flowers are infected per day in this group of epidemics.

There was no significant difference between the LIG group and the other groups of epidemics with respect to RRFS (relative rate of flower senescence, mean value of 0.076 flower • flower^{-1} • day^{-1}; Table 7). However, this group was characterized by a high RRRF (relative rate of removed flowers, with a mean of 0.114 flower • flower^{-1} • day^{-1}) (Table 7).

LIG has an average RRP (relative rate of primary infection on foliage) value of 0.02 plant • plant^{-1} • day^{-1} (Table 7). However, LIG presented a RRS (relative rate of secondary infection) mean value of 0.05 plant • plant^{-1} • day^{-1} (Table 7).

The moderate intensity epidemic group (MIG) is a group of white mould epidemics with AUDPCs ranging between 731 and 2193 diseased plant.days. This group is composed of eighty-six white mould epidemics (Table 5). With respect to SC (number of sclerotia), MIG has an average SC value of 527 sclerotia in the simulated system (Table 5), which is higher than the low (LIG), but smaller than the high disease intensity group. MIG is also characterised by a higher RRGS (relative rate of germinated sclerotia) than the low intensity epidemic group (LIG), with a value of 0.094 sclerotia • sclerotia^{-1} • day^{-1} ($P < 0.01$) (Table 5). However, MIG did not significantly differed from the other groups when considering the mean RRDS (relative rate of death of sclerotia) and RRFS (relative rate of flower senescence), (Table 7).

The MIG group has an average RRFI (relative rate of flower infection) value of 0.099 flower • flower^{-1} • day^{-1} (Table 7), that is, 9.9% of healthy flowers are infected per day in this group. MIG is also characterized by a high relative rate of removed flowers (RRRF), with a value of 0.107 flower • flower^{-1} • day^{-1} (Table 7). RRP (relative rate of primary infection on foliage) is 0.019 plant • plant^{-1} • day^{-1} (Table 7). RRP did not show statistical difference between MIG and LIG, but is lower than in the last, high intensity, epidemic group. Concerning the relative rate of secondary infection on foliage (RRS), MIG presented an RRS of 0.051 plant • plant^{-1} • day^{-1}, intermediate between LIG and HIG (Table 7).

The high intensity white mould epidemic group (HIG) has AUDPCs greater than 2193 diseased plant • days. This group is composed of fourteen simulated white mould epidemics (Table 7). HIG is characterized by the highest SC (number of sclerotia in the system) ($P <$ 0.05) (Table 7). This group has an SC value of 679 sclerotia per unit area. HIG also has the highest RRGS (relative rate of germinated sclerotia per unit area) with of 0.111 sclerotia • sclerotia^{-1} • day^{-1} (Table 7).

HIG presents the highest RRFI (relative rate of flower infection) value, which differs significantly from LIG, with 0.111 flower • flower^{-1} • day^{-1}, that is, 11.1% of healthy flowers are infected per day in this high intensity epidemic group. This group also is characterized by the lowest RRRF (relative rate of removed flowers), with 0.071 flower • flower^{-1} • day^{-1} ($P <$ 0.05) (Table 7). In addition, HIG presents the highest RRP (relative rate of primary infection on foliage) value of 0.026 plant• plant^{-1} • day^{-1} ($P <$ 0.05) (Table 7). HIG is also characterized by a RRS (relative rate of secondary infection on foliage) of 0.057 plant • plant^{-1} • day^{-1}, which is higher than the low intensity epidemic group (Table 7), but did not differ from MIG.

3.4 Discriminant analysis of epidemic intensity groups and Wilk's Lambda

Eight canonical discriminant factors (Table 8) were extracted through discriminant analysis (Figure 9, Table 8) of the 144 simulated bean white mould epidemics.

A jackknifed classification matrix using the eight model parameters (SC, RRGS, RRDS, RRFI, RRFS, RRRF, RRP and RRS) correctly characterised 84% of the three epidemic groups (Table 9). With respect to the low intensity epidemic group (LIG), the discriminant function correctly characterized 95% of the simulated epidemics, as all 44 simulated white mould epidemics are correctly classified in LIG (Table 9; Figure 9). Two white mould epidemics are incorrectly classified in the moderate intensity epidemic group (MIG), and no epidemic is incorrectly classified in the high intensity epidemic group (HIG) (Table 9).

Regarding the MIG, the discriminant function correctly characterizes 78% of white mould epidemics. Sixty-seven out of 86 simulated white mould epidemics are correctly classified as MIG (Table 9; Figure 9). Six white mould epidemics are incorrectly classified in HIG and 13 epidemics are incorrectly classified as LIG (Table 9).

With respect to the high intensity epidemic group (HIG), the discriminant function correctly characterised 86% of white mould epidemics. Twelve out of 14 simulated white mould epidemics were correctly placed in HIG (Table 9; Figure 9). Two white mould epidemics were incorrectly placed in MIG and no epidemics were incorrectly categorized as LIG (Table 9).

The Wilk's Lambda statistic was employed to test for dispersion among all the groups on all the variables. Lambda varies from 0 to 1.0 with 0 indicating that group means differ, and 1 meaning that all group means are the same (Engelman, 2009). A Wilks's Lambda value of 0.223 indicated that group means differ ($P < 0.01$).

4. DISCUSSION

Construction of an ethograph (Figure 1), followed by the simplified overall structure of the disease cycle were instrumental for the conceptualization and development of this simulation model. Based on these structures, a preliminary simulation model for white mould epidemics was built, which enabled identifying the main processes involved on the production of each one of the four kinds of propagules. Figure 2b represents the structure of the preliminary simulation model for white mould epidemics on bean. This simplified model was achieved by the replacement of some sub-processes of the disease cycle by their respective relative rates of infection. Simplification of the model was highly desirable in order to reduce the number of parameters, enabling the development of a useful, workable model. When the ethograph (Figure 2b) is compared with the developed model, a clear structural analogy between them is noted.

Inspection of the shapes of simulated disease progress curves, and, especially, comparison of actual and simulated curves indicate if one model adequately represents real life situations. The shape of the simulated epidemic progress curves of this model showed patterns similar to those observed previously in white mould epidemics in the field (Boland & Hall, 1987). Similar patterns were also observed on sclerotinia flower blight epidemics in a Perennial Pyrethrum (Pethybridge et al., 2010, Scott et al., 2013), lettuce drop epidemics (Hao & Subbarao, 2005) and sclerotinia rot of carrot (Kora et al., 2005). The structure of the white mould model is based on three sub-models, representing three groups of processes. The first sub-model (sub-model 1) considers processes involved in the sclerotia germination; the second (sub-model 2) encompasses processes related to bean flower infections; the third (sub-model 3) accounts for processes involved in the bean foliage infections.

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AUDPC is an output of white mould model that integrates a large amount of information into one variable (Campbell & Madden, 1990a, Cooke, 2006) . AUDPC was chosen because it aggregates information from the whole duration of the epidemic course, materialized by an epidemic curve representing incidence versus time. However, AUDPC does not carry information on the shape of the disease curve, which may be a limitation of this variable (Campbell & Madden, 1990a, Cooke, 2006).

Sensitivity analyses allowed the estimation of the magnitude of effects of each parameter on epidemic intensities. Sensitivity analysis of sub-model 1 indicated that the amount of sclerotia (SC) was the most influential parameter of the intensity of the simulated epidemics, as measured by the AUDPC. An increase of the SC parameter caused a very strong increase in the area under disease progress curve (AUDPC). The second most important parameter of sub-model 1 was the relative rate of germination of sclerotia (RRGS). Since the parameter relative rate of death of sclerotia (RRDS) showed lower sensitivity, it lead to a smaller variation of the AUDPCs in the simulated epidemics.

Sensitivity analysis of sub-model 2 showed that the relative rate of flower infection (RRFI) was the most important parameter for explaining the simulated epidemic intensities. Variation of RRFI resulted in a greater effect on the variation in the area under incidence progress curve than the variation of the relative rate of flower senescence (RRFS). The relative rate of removed flowers (RRRF) was the parameter that caused the smallest average changes in the AUDPCs of the simulated epidemics. These results were confirmed by the significance of these parameters in the analyses of variance according to the groups (Table 6) and in the mean separation tests (Table 9).

In sub-model 3, which simulates infection of the bean foliage, the relative rate of primary infection of the foliage (RRP) was the parameter that produced the greatest variation in AUDPC. The relative rate of secondary infection (RRS) caused a smaller amount of variation in AUDPC than RRP. However, when actual white mould epidemics are compared with these simulated epidemics, it appears that the range value of RRS parameters was probably underestimated in the simulated epidemics. The relative small variation in AUDPC detected due to RRS variation suggests that the range value of RRS was probably underestimated due to the very limited number of propagules able to cause secondary infection: all estimates were made based on a maximum number of propagule 4 (infectious hyphae), corresponding to 50 plants, which may be considered very limited. On the other hand, variation in the number of primary infections on foliage showed a higher number of propagules to cause infections on foliage, since, in this case, a propagule corresponds to one infected flower (propagule 3) and the maximum number of propagule 3 was set as 1500 flowers, which is a much larger range than the range used for RRS.

We propose that the rate of secondary infection on white mould epidemics may be more relevant and complex than currently depicted. The rate of secondary infection is proportional to the amount of infected tissues and also depends on the amount of tissues still available for infection (Savary, 1996, Savary et al., 1997). Similar structure of secondary infection was proposed by Savary et al. (1997) for sheath blight epidemics where secondary spread takes place through direct contact between healthy and infected tissues. Web blight of beans caused by *Thanatephorus cucumeris* is also spread through mycelial bridges (Costa-Coelho et al., 2014). Aghajai et al. (2010) also showed that the secondary spread of sclerotinia stem rot of canola epidemics occurs via plant contacts.

Actual field observations indicate that severe white mould epidemics involve amounts of lesions per plant that are much higher than in the least severe epidemics (Data shown in Chapter 2).

Infected plants with a great number of lesions provide a much larger chance of infecting healthy plants, than infected plants with only one lesion. However, although propagule 4 was set to represent the infectious hyphae actively growing in infected host tissue, the estimation of this variable was made by the number of infected plants. This may be a good approximation of the conceptual value of propagule 4 for white mould epidemics, when disease intensities are low, with few lesions per plant. Nevertheless, in severe white mould epidemics, when one plant may have many lesions, it may underestimate the value of the rate of secondary infections. Thus, RRS may be most important in severe epidemic situations. The concepts of the infection cycle were presented by Bergamin Filho and Amorim (2001) when discussing the concept of an anti-clockwise flow of inoculum by growth of existing lesions. This concept was actually taken into account in designing this white mould simulation model when I considered that the secondary infection on foliage may develop from spread of mycelium (infectious hyphae) from diseased tissues to the healthy ones (propagule 4).

Forecasts of white mould epidemics have been developed based on petal infestation during early bloom (Turkington et al., 1991). These forecasts were relatively accurate when disease risk and incidence were low, but less so when disease risk and incidence were moderate to high. This suggests that when disease incidence is high (i.e., when the proportion of diseased plants is high) secondary infections (plant to plant infections) have a

strong effect on the dynamics of epidemics, so that forecast of white mould based on petal infestation becomes insufficient.

The different analyses conducted (analyses of variance and principal component analysis) indicate that SC, RRGS, RRFI, RRP and RRS were the most important parameters of this white mould model. Our results indicate that, among these five parameters (out of eight considered in our model), some are especially important in determining whether an epidemic will be weak or moderate, while other parameters are associated with strong epidemics, as opposed to moderate ones. In short, the characterization of three epidemic groups through cluster analysis was generated by the combination of parameters values of the white mould model. Parameters that distinguish LIG from MIG were SC, RRGSC and RRFI. Thus, epidemics with low quantity of sclerotia in the soil (SC), low relative rate of germinated sclerotia (RRGSC), along with a low relative rate of flower infection (RRFI), are classified as low intensity epidemics. The above three parameters were most important in discriminating LIG from MIG.

High intensity epidemics differed from the MIG by the RRP, RRRF and SC parameters ($P < 0.05$) (Table 7). Therefore, HIG becomes different from MIG by increases in the relative rate of primary infection on foliage (RRP), reduction of the relative rate of removed flowers and also by a higher quantity of sclerotia in the system.

Low intensity epidemics are different from the epidemics classified in the HIG by a large number of parameters (SC, RRGSC, RRFI, RRRF, RRP e RRS) (Table 7). Therefore, a great number of parameters determine whether a high intensity epidemics can develop. For a HIG epidemic to take place, it is necessary a high quantity of SC, a greater RRGSC, a high RRFI, a low RRRF and also increases in RRP and RRS.

The Low Intensity Group (LIG) of epidemics, with smaller AUDPC values, was positioned mainly on the left part of the discriminant analysis graph (Figure 8), corresponding to larger relative rates of removed sclerotia, flower senescence, and removed flowers, and low numbers of sclerotia in the system, relative rates of germinated sclerotia, infected flowers, and primary infection on foliage (Figure 7).

Therefore, RRDS and RRFS have not shown significant contributions to the formation of epidemic groups (Table 7). These results were confirmed both by the analyses of variance as by the sensitivity analysis and the discriminant functions. This result does not imply that these parameters do not affect white mould epidemics in the field, but, rather, that they do not sufficiently affect the epidemic progress to be useful in separating the groups.

High relative rates of flower infection favour higher disease incidences and consequently higher AUDPCs. Increased relative rates of sclerotia death were linked to epidemics with lower disease incidences. Similarly, increased relative rates of flower senescence were associated with epidemics with lower disease incidences. Fusarium head blight (FHB) of wheat is an important disease that follows this pattern, and also the ascospores are able to infect the wheat heads only in a certain short period namely during anthesis stage, as white mould on beans (Fernando et al., 1997).

Discriminant analysis showed that 84% of epidemic groups were correctly classified by the simulation model we developed. These results were obtained using the eight model parameters in the discriminant analysis (SC, RRGSC, RRDS, RRFI, RRFS, RRRF, RRP and RRS). The parameters were efficient for discriminant functions (Table 8). Low intensity epidemics were characterized by low final disease incidences and low amounts of germinated sclerotia (GSC). This group also presented a low amount of infected flowers (Figure 10). The

moderate intensity epidemics group had larger quantities of germinated sclerotia than LIG. This group also showed a larger quantity of infected flowers than LIG. Consequently, the Moderate group showed had a higher final disease incidence than group Low. The final disease incidence in group HIG was the highest among all groups, with 100% of the plants infected. As in the sensitivity analysis, the discriminant functions revealed that the amount of sclerotia per microplot (SC) was the most important parameter to categorise the epidemic groups (0.988). These results agree with the published literature on white mould, in that the most efficient method of control is to prevent the entry or to reduce the number of sclerotia structures (SC) in the field (Coley-Smith & Cooke, 1971, Young et al., 2004).

The relative rate of flower infection (RRFI) is the second most important parameter in discriminant function 1 with a value of 0.864. This suggests that other important control measures should prioritize the reduction of the relative rate of flower infections (RRFI). Thus the most commonly used control measure is the application of fungicides at the flowering stage (Oliveira et al., 1999, Vieira et al., 2012). In addition, measures such as increasing plant row spacing will reduce relative humidity in the canopy, leaf wetness duration and consequently, impair flower infection by ascospores (Clarkson et al., 2003, Vieira et al., 2010). Breeding for more upright plant architecture will achieve the same end. Coupled with these control measures, the reduction of the relative rate of primary infection on foliage (RRP) was also shown to be very important in this disease model. Here, the application of fungicides is also the most commonly used control measure. There are several other control measures which can act to reduce the two parameters values (RRFI and RRP). For instance plant row spacing and plant architecture (Schwartz & Steadman, 1978, Boland & Hall, 1988a, Boland & Hall, 1988b, Lobo Junior et al., 2009).

The relative rate of germinated sclerotia (RRGSC) is the third most important parameter in the discriminant function 1 with a value of 0.608. The results showed that to reduce the relative rate of sclerotia germination is also another efficient measure in the white mould control (Tu, 1989b, Macena et al., 2011).

Concerning the discriminant function 2, the relative rate of primary infection on foliage was the most important parameter. Tu (1989a) observed that the majority (78%) of bean plant infections were provided by infected petals. However, relative rate of secondary infection (RRS) showed a larger importance in the discriminant function 2 with a value of 0.219 than in the discriminant function 1 with a value of 0.197.

Bean white mould is a notoriously difficult disease to manage, and the difficulty in predicting the outcome of epidemics is well known (Vieira et al., 2012). The development of a mechanistic model for bean white mould epidemics, emphasizing the successive types of pathogen propagules during the disease cycle and their interaction with host plants represent an advance to help in the understanding of the disease. The process of conceptualization of the disease process, structure simplification, and the definition of the most important parameters resulted in a robust working model for understanding the dynamics of epidemics in this pathosystem. Foremost, this work overcomes the limitations usually associated with treatment of this disease as a simple "monocyclic" disease. Clearly, this pathosystem is much more complex than the theoretical general "simple interest" diseases model, even if the amount of initial inoculum (in the form of propagule 1, sclerotium) remains as one of the most important parameters, as shown in the sensitivity analysis. Improvements of the model may include further studies on the variation of the RRS

parameter relative rate of contact infection (or relative rate of secondary infection) (sub-model 3) to match more closely the actual disease dynamics in high intensity epidemics.

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Figures
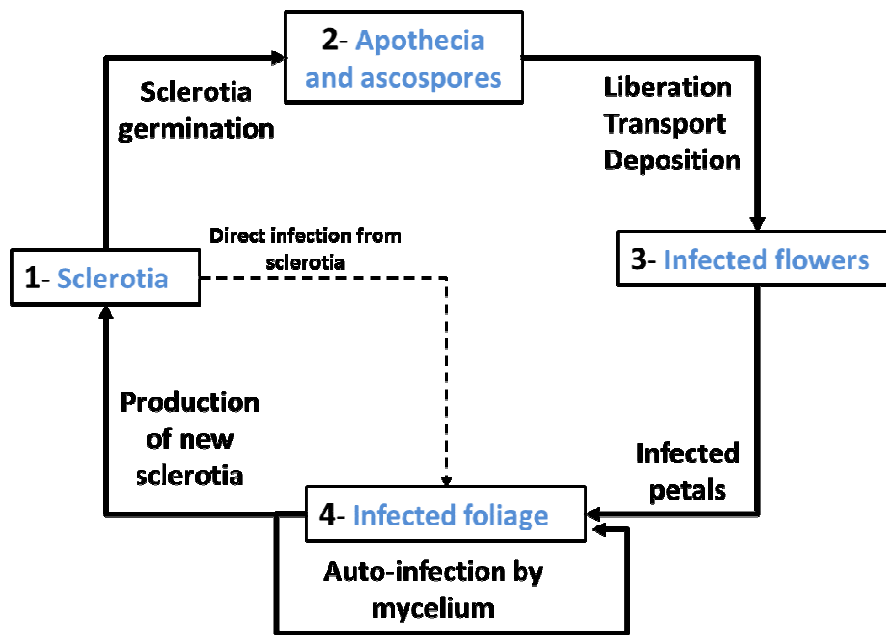
Figure 1. Generalized ethograph of white mould (*Sclerotinia sclerotiorum)* cycle showing relationships between pathogen propagules. Squares represent the four types of pathogen propagules. Continuous arrows represent most important flows of infection. Discontinuous arrows represent flows of infections with low frequency. Blacktext represents processes between pathogen propagules. 1- The first pathogen propagule type corresponds to sclerotia. 2- Apothecia and ascospores represent the second type of propagule. 3-Infected petals are the third type of pathogen propagule. 4- Infectious hyphae in the host foliage represent the fourth type of propagule.
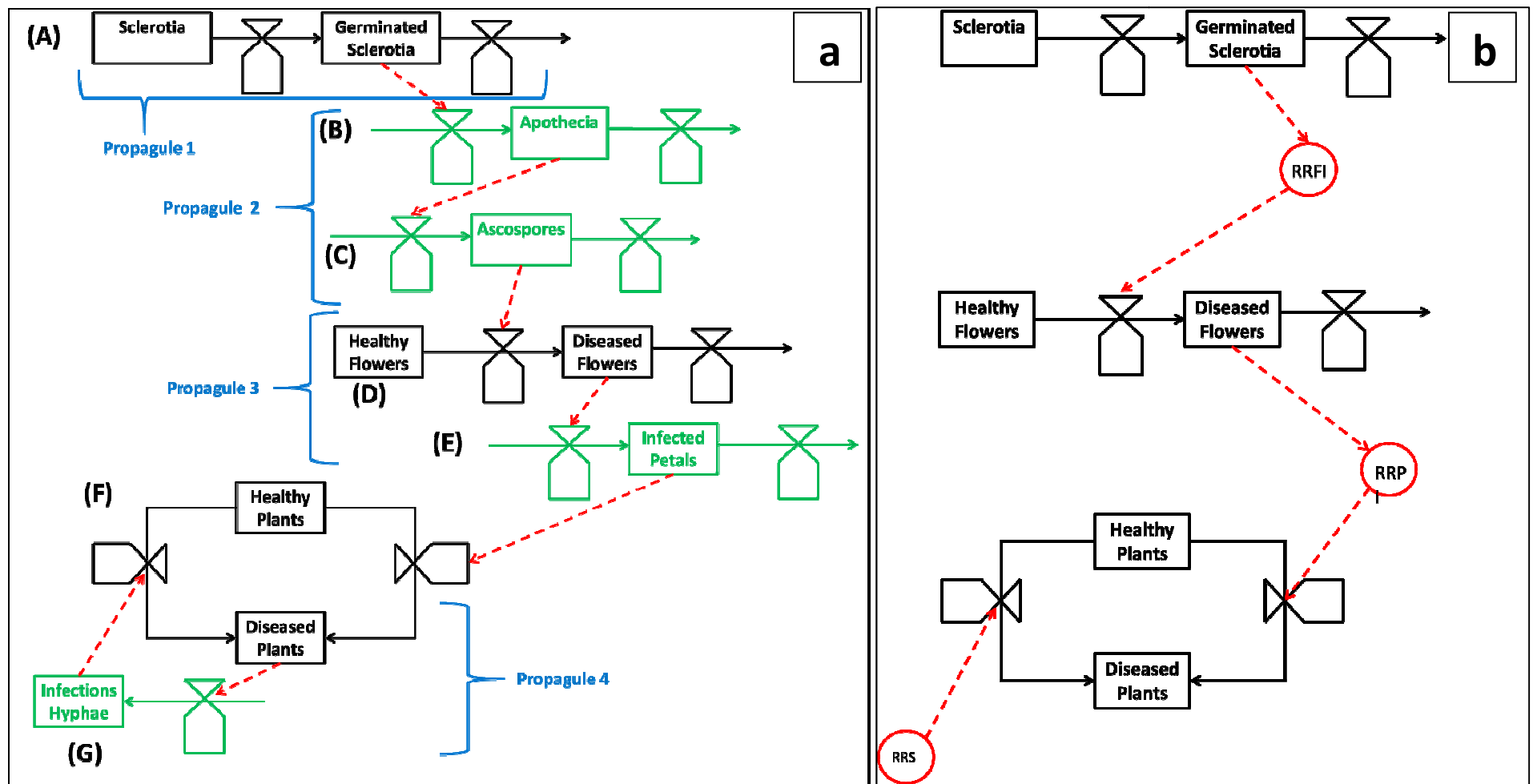
Figure 2. (**a**)- Representation of the overall structure of disease cycle for white mould epidemics of bean synthesised as sub-processes A, B, C, D, E, F and G as a function of propagule type (Propagule 1, propagule 2, propagule 3 and propagule 4). (**b**)- Representation of the simplified structure of a preliminary simulation model for white mould epidemics. Squaresare state variables;Red circles; are relative rates; ⛏ are rates

of transfer from one state variable to another. Continuous arrows represent flows. Discontinuous arrows represent flows for links between two sub-models. In overall structure of disease cycle: **A**- represents sclerotia germination; **B**- characterises apothecia formation; **C**- represents production, liberation, transport and deposition of ascospores; **D**- denotes infection of flowers; **E**- symbolizes infected petals; **F**- represents primary and secondary infection on foliage; **G**- characterises infection hyphae plant to plant. Green represents sub-processes that were simplified by relative rates (red circles) in the preliminary simulation model in (b): **RRFI**- relative rate of flower infection; **RRP**- relative rate of primary infection on foliage; **RRS**- relative rate of secondary infection on foliage.
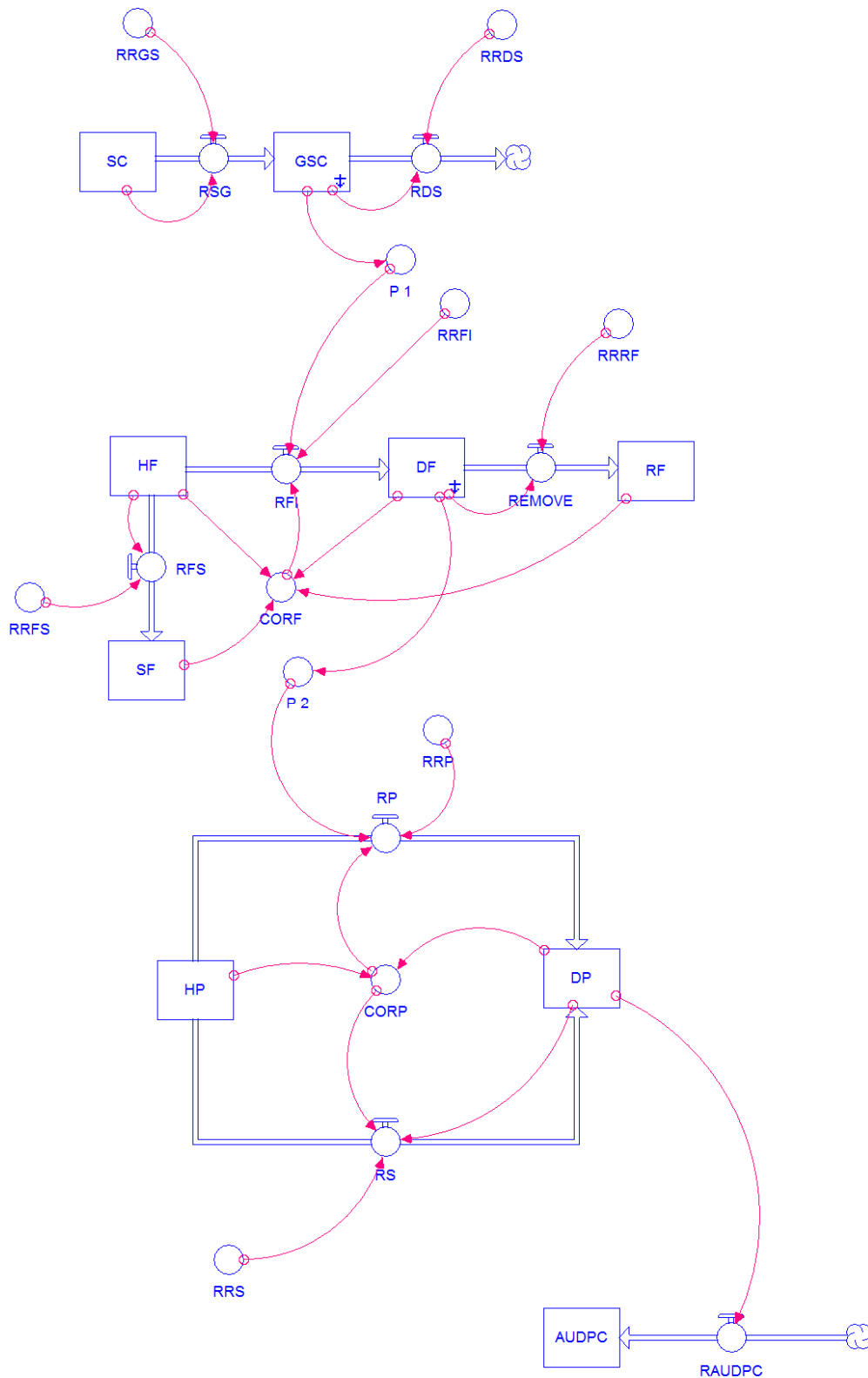
Figure 3. Overall structure of a preliminary simulation model for bean white mould epidemics. This structure is divided into four parts that are functions of the disease propagules. Boxes indicate state variables, circles indicate parameters, double arrows indicate flows of individuals (Plants or propagules), and simple arrows indicate numerical

relationships. Two flows and their rates indicate two processes of infection, converting healthy plants (HP) into diseased ones (DP). **HP**- healthy plants; **DP**- diseased plants; **RP**- rate of primary infection on foliage; **RRP**- relative rate of primary infection on foliage; **RS**- rate of secondary infection on foliage; **RRS**- relative rate of secondary infection on foliage; **CORF**- correction factor for flower infection; **CORP**- correction factor for plant infection; **SC**- Sclerotia; **GSC**- Germinated sclerotia; **RGS**- rate of germinated sclerotia; **RRGS**- relative rate of germinated sclerotia; **RDS**- rate of death of sclerotia; **RRDS**- relative rate of death of sclerotia; **HF**- healthy flowers; **DF**- diseased flowers; **RFI**- rate of flower infection; **RRFI**- relative rate of flower infection; **RFS**- rate of flower senescence; **RRFS**- relative rate of flower senescence; **RRF**- rate of removed flowers; **RRRF**- relative rate of removed flowers; **AUDPC**- area under disease progress curve; **RAUDPC**- rate of area under disease progress curve. See Table 3.1 for the meaning of symbols and dimensions.
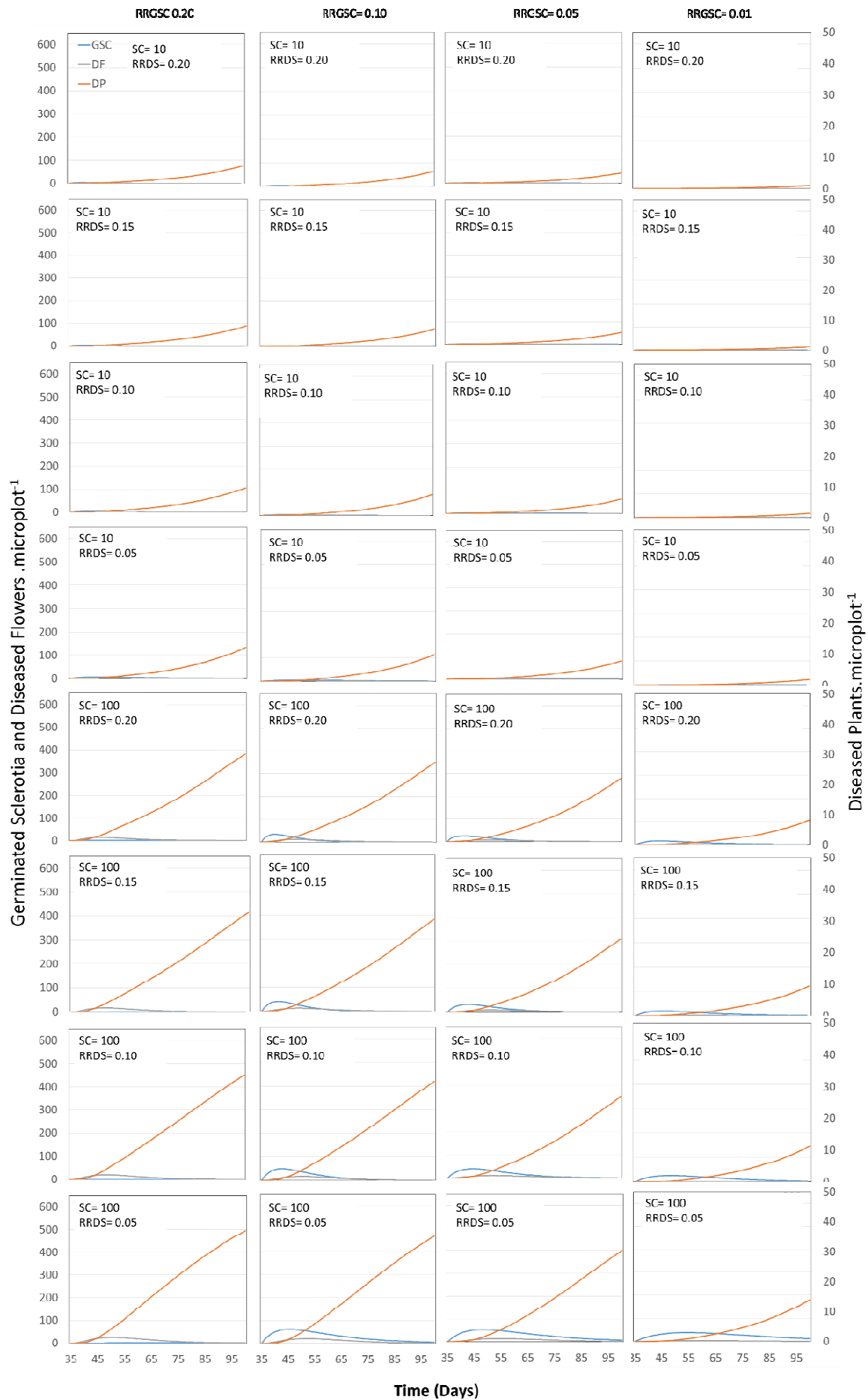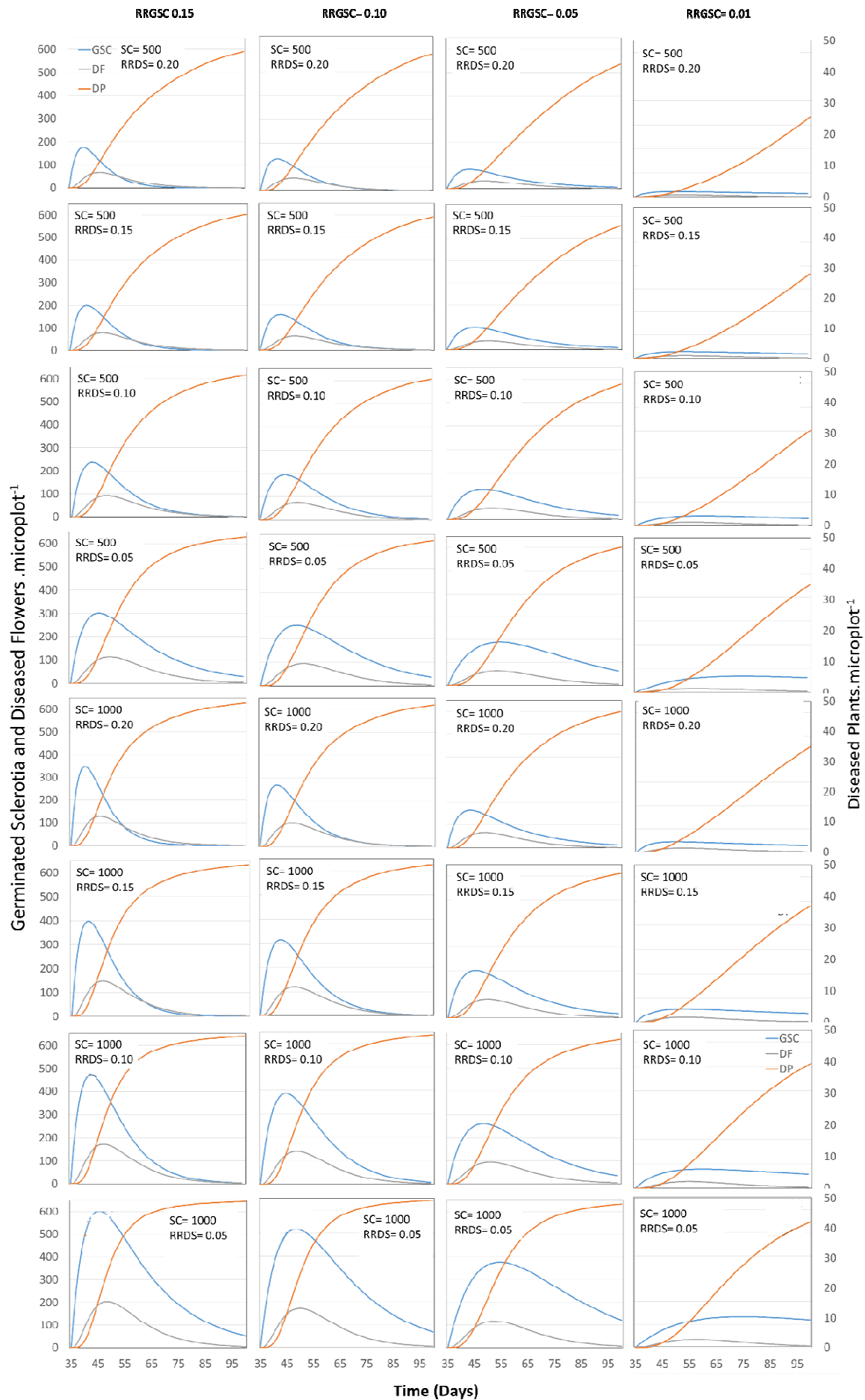
Figure 4. Outputs of a sensitivity analysis of a white mould simulation model, considering the sclerotia germination sub-model. Abscissa: elapsed time (days). Ordinates: Left = number of propagules per microplot ($5m^2$); Right = number of diseased plants per microplot ($5m^2$). GSC- germinated sclerotia; DF- diseased flowers; DP- diseased plants. All parameters within the sclerotia germination sub-model were set vary on four levels: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death sclerotia. The analysis was carried with 64 simulated white mould epidemics: 4 values of SC (initial) * 4 values of RRDS * 4 values of RRGSC.
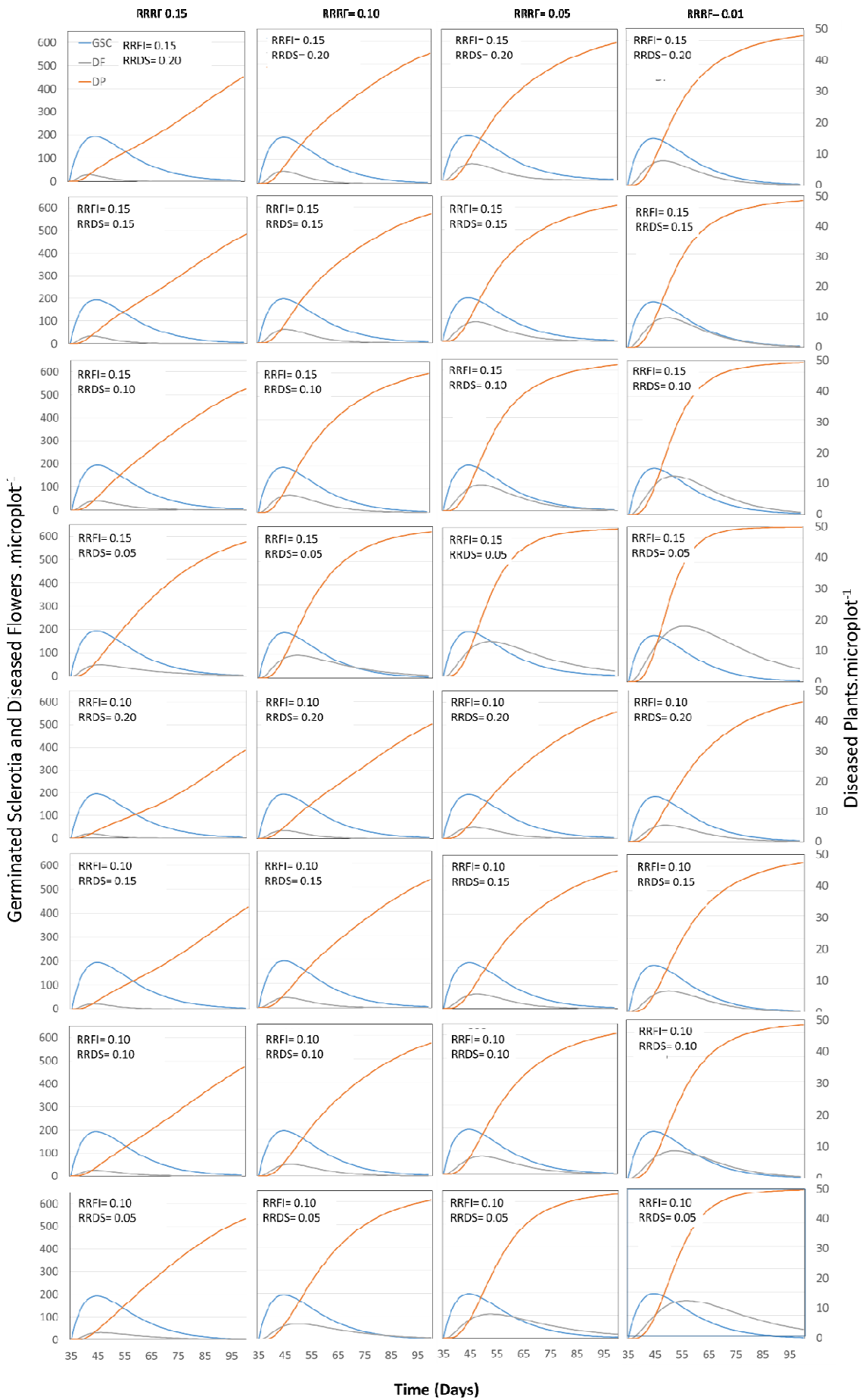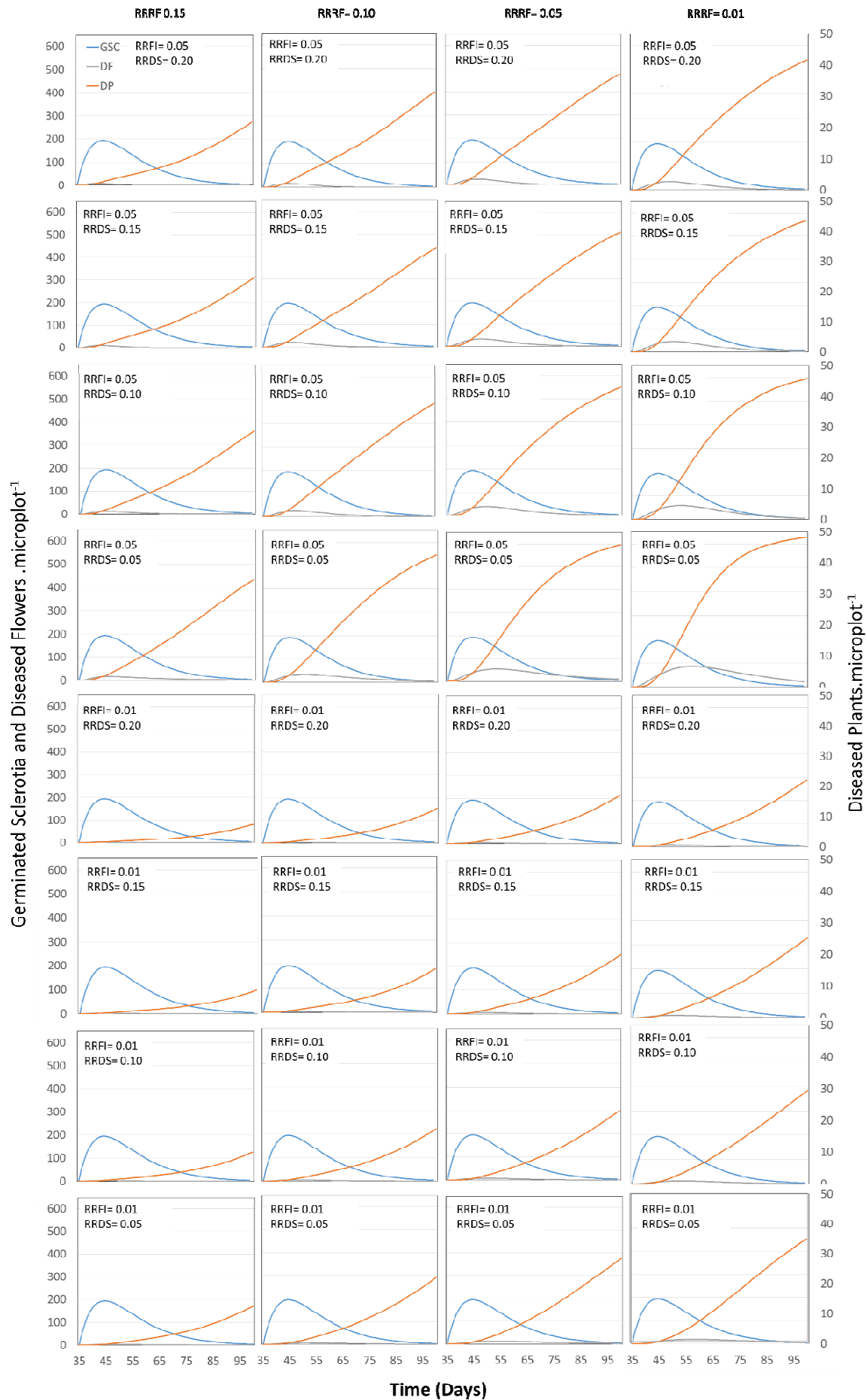
Figure 5. Outputs of a sensitivity analysis of a white mould simulation model, considering the flower infection sub-model. Abscissa: elapsed time (days). Ordinates: Left = number of propagules per microplot (5m^2); Right = number of diseased plants per microplot (5m^2). GSC- germinated sclerotia; DF- diseased flowers; DP- diseased plants. All parameters within the flower infection sub-model were set vary on four levels: RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers. The analysis was carried with 64 simulated white mould epidemics: 4 values of RRFI* 4 values of RRFS * 4 values of RRRF.
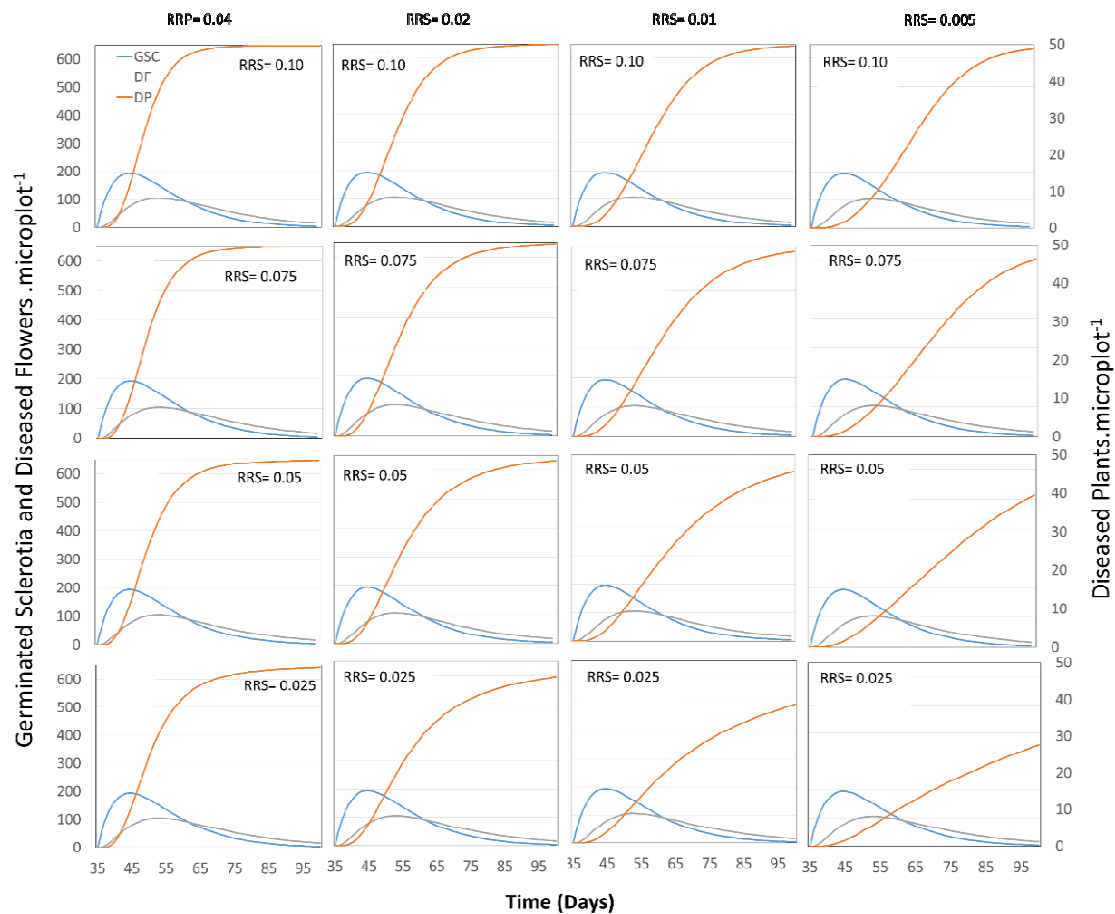
Figure 6. Outputs of a sensitivity analysis of a white mould simulation model, considering the foliage infection sub-model. Abscissa: elapsed time (days). Ordinates: Left = number of propagules per microplot (5m^2); Right = number of diseased plants per microplot (5m^2). GSC- germinated sclerotia; DF- diseased flowers; DP- diseased plants. The two parameters within the foliage infection sub-model were set vary on four levels: RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage.   The analysis was carried with 16 simulated white mould epidemics: 4 values of RRP * 4 values of RRS.
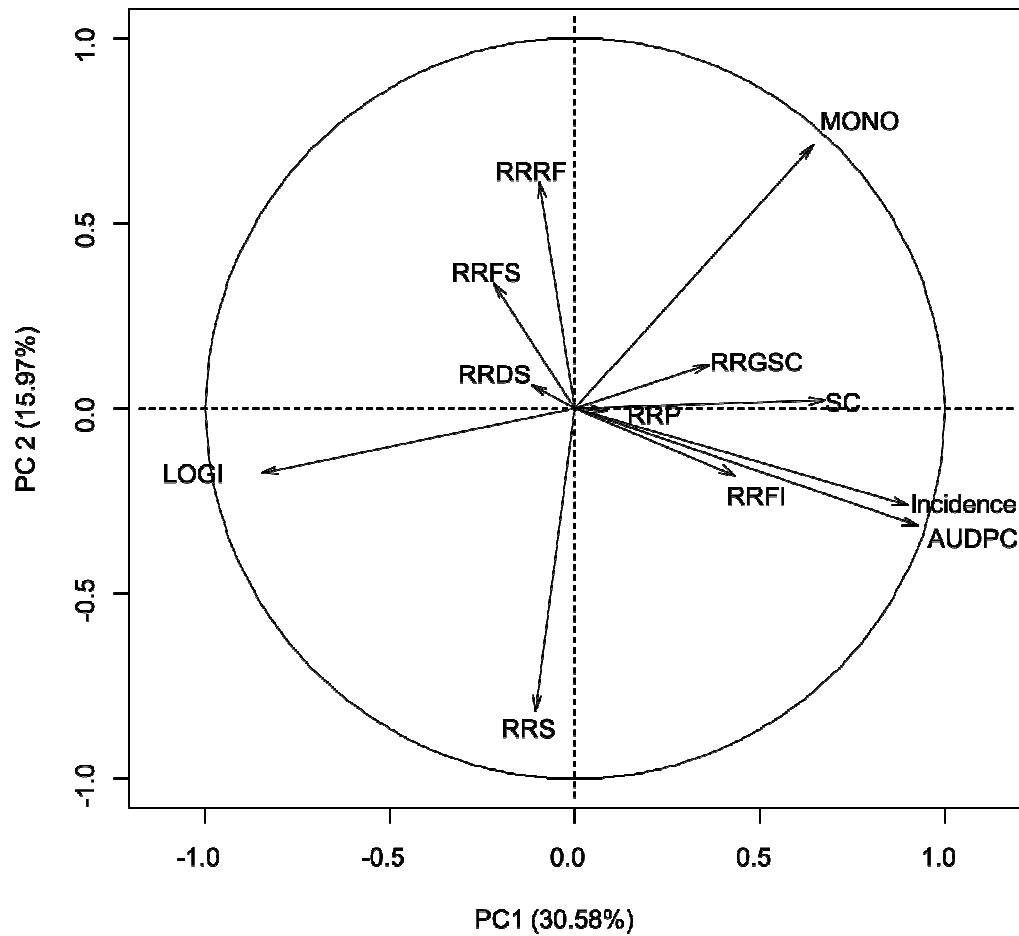
Figure 7. Principal component analysis of 144 simulated white mould epidemics performed using the number of sclerotia on soil per 5 m^2 of dry beans (SC), relative rate of germinated sclerotia (RRGS), relative rate of death of sclerotia (RRDS), relative rate of flower infection (RRFI), relative rate of flower senescence (RRFS), relative rate of removed flowers (RRRF), relative rate of primary infection on foliage (RRP) and relative rate of secondary infection on foliage (RRS), coefficients of determination of the temporal analyses of monomolecular (MONO) and logistic models (LOGI). PC1 refers to the first component and PC2 refers to the second component.
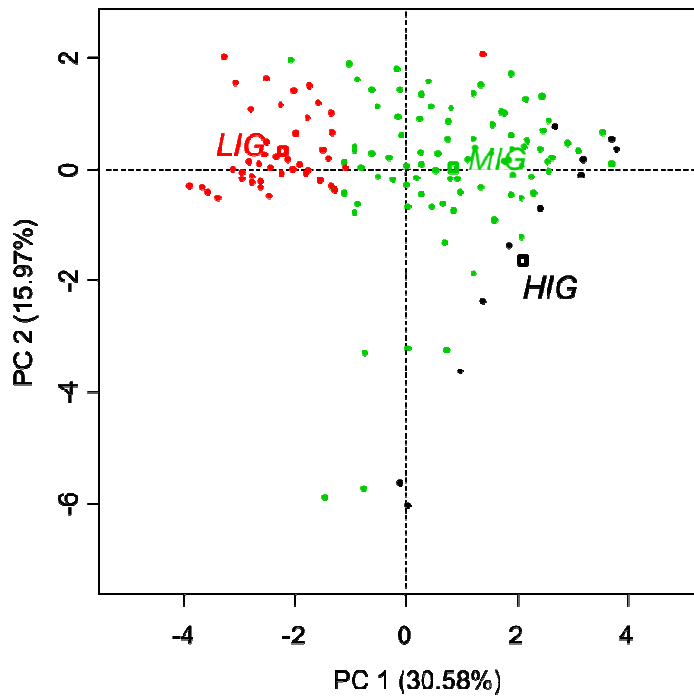
Figure 8. Principal component analysis showing each of 144 simulated white mould epidemics and respectively groups originated from AUDPC value levels. This analysis was performed using the number of sclerotia on soil per 5 m^2 of dry beans (SC), relative rate of germinated sclerotia (RRGS), relative rate of death of sclerotia (RRDS), relative rate of flower infection (RRFI), relative rate of flower senescence (RRFS), relative rate of removed flowers (RRRF), relative rate of primary infection on foliage (RRP) and relative rate of secondary infection on foliage (RRS). PC1 refers to the first component and PC2 refers to the second component.
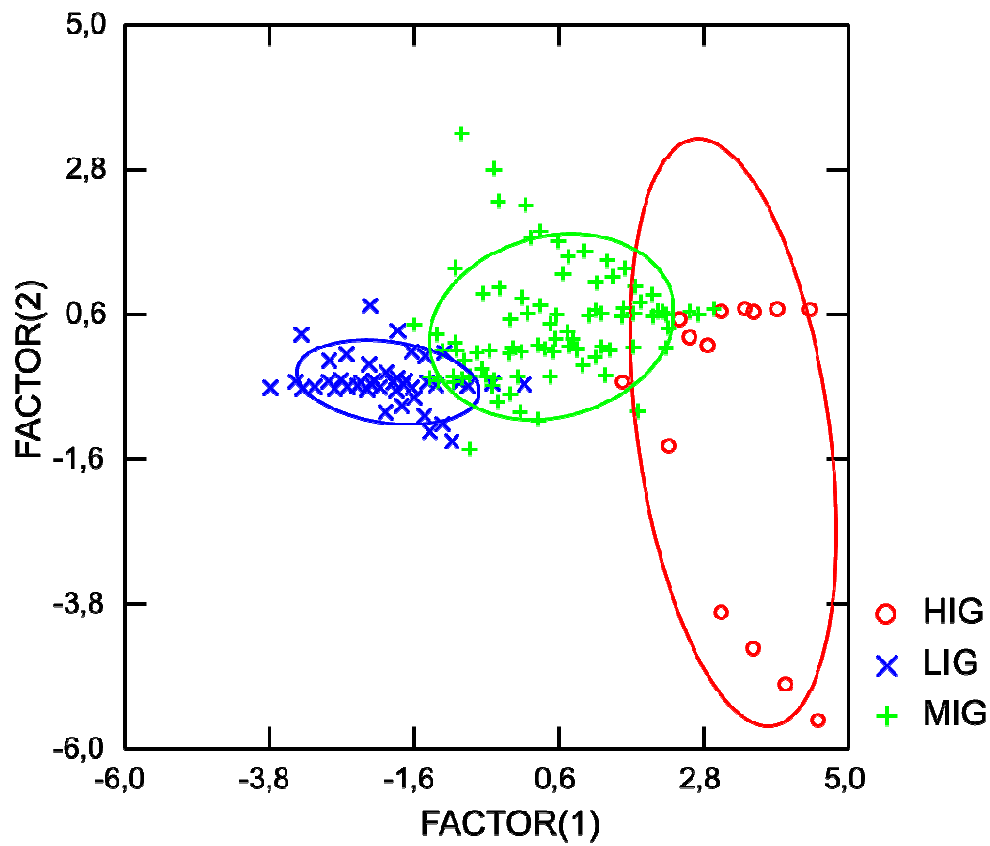
Figure 9. Discriminant analysis of the model parameters in function of groups generated from calculations of respective areas under incidence progress curves. Discriminant analysis was performed with 144 simulated white mould epidemics using eight parameters of white mould model: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of infected flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage.
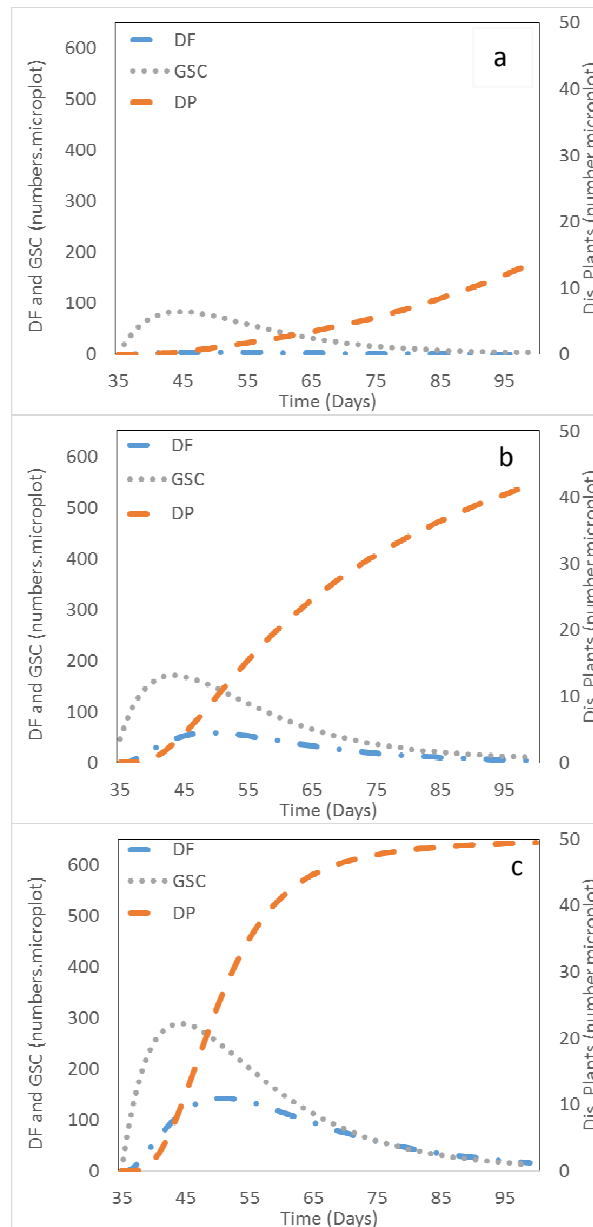
Figure 10. Epidemic groups generated from area under incidence progress curve of 144 simulated white mould epidemics. **a**: average parameter dynamics of Low Intensity Group from 44 white mould epidemics; **b:** average parameter dynamics of group Moderate Intensity Group from 86 white mould epidemics; and **c:** average dynamics of group High Intensity Group from 14 white mould epidemics. Each graph show three outputs of bean white mould model: DF-Diseased flowers per microplot (5m²); GSC-Germinated sclerotia; DP-Diseased plants. Abscissa: elapsed time (days). Ordinates: Left = number of propagules per microplot (5m²); Right = number of diseased plants per microplot (5m²).

Tables

Table 1. List of state variables, rates and parameters described on the bean white mould model

Symbol	Meaning of symbol	Dimensions[a]	Unit
State variables			
SC	Number of sclerotia on soil (per 5 m^2 of dry beans)	[N$_{sclerotia}$]	sclerotia
GSC	Number of germinated sclerotia (per 5 m^2 of dry beans)	[N$_{sclerotia}$]	sclerotia
SCD	Number of dead sclerotia (per 5 m^2 of dry beans)	[N$_{sclerotia}$]	sclerotia
HF	Number of healthy flowers (per 5 m^2 of dry beans)	[N$_{flowers}$]	flower
DF	Number of diseased flowers(per 5 m^2 of dry beans)	[N$_{flowers}$]	flower
HP	Number of healthy plants (per 5 m^2 of dry beans)	[N$_{plants}$]	plant
DP	Number of diseased plants (per 5 m^2 of dry beans)	[N$_{plants}$]	plant
Rates			
RSG	Rate of sclerotia germination	[N$_{sclerotia}$ T^{-1}]	sclerotia.day^{-1}
RSD	Rate of removal sclerotia	[N$_{sclerotia}$ T^{-1}]	sclerotia.day^{-1}
RFI	Rate of flowers infection	[N$_{flowers}$ T^{-1}]	flower.day^{-1}
RRF	Rate of removed flowers	[N$_{flowers}$ T^{-1}]	flower.day^{-1}
RFS	Rate of flowers senescence	[N$_{flowers}$ T^{-1}]	flower.day^{-1}
RP	Rate of petal infection	[N$_{plants}$ T^{-1}]	plant.day^{-1}
RS	Rate of contact infection	[N$_{plants}$ T^{-1}]	plant.day^{-1}
Parameters & coefficients			
CORF	Correction factor of flowers infection	[-]	healthy flowers.total flowers^{-1}
CORP	Correlation factor of plant infection	[-]	healthy plants.total plants^{-1}
RRSG	Relative rate of sclerotia germination	[T^{-1}]	sclerotia. sclerotia^{-1}.day^{-1}
RRSD	Relative rate of removal sclerotia	[T^{-1}]	sclerotia. sclerotia^{-1}.day^{-1}
RRFI	Relative rate of flowers infection	[T^{-1}]	flower. flower^{-1}.day^{-1}
RRFS	Relative rate of flowers senescence	[T^{-1}]	flower. flower^{-1}.day^{-1}
RRRF	Relative rate removed flowers	[T^{-1}]	flower. flower^{-1}.day^{-1}

RRP		Relative rate of primary infection on foliage	$[T^{-1}]$	plant. $plant^{-1}.day^{-1}$	
RRS		Relative rate of secondary infection	$[T^{-1}]$	plant. $plant^{-1}.day^{-1}$	

Table 2. Parameter values of white mould model that were used for sensitivity analyses for each sub-model. Parameters were set to vary within four levels. For each sub-model evaluation, the remaining parameters were fixed

Sub-model	Symbol	Meaning of symbol	Parameters variation	Unit	Fixed parameters
Sclerotia germination	SC	Number of sclerotia on soil (per 5 m^2 of dry beans)	10, 100, 500 or 1000	sclerotia	RRFI=0.10; RRFS=0.05; RRRF= 0.10; RRP=0.02; RRS=0.05
	RRSG	Relative rate of sclerotia germination	0.15, 0.10, 0.05 or 0.01	sclerotia. $sclerotia^{-1}.day^{-1}$	
	RRSD	Relative rate of removal sclerotia	0.20, 0.15, 0.10 or 0.05	sclerotia. $sclerotia^{-1}.day^{-1}$	
Flower infection	RRFI	Relative rate of flowers infection	0.15, 0.10, 0.05 or 0.01	flower. $flower^{-1}.day^{-1}$	SC=500; RRGS=0.10; RRDS=0.10; RRP=0.02; RRS=0.05
	RRFS	Relative rate of flowers senescence	0.20, 0.15, 0.10 or 0.05	flower. $flower^{-1}.day^{-1}$	
	RRRF	Relative rate removed flowers	0.20, 0.10, 0.05 or 0.01	flower. $flower^{-1}.day^{-1}$	
Foliage infection	RRP	Relative rate of primary infection on foliage	0.04, 0.02, 0.01 or 0.005	plant. $plant^{-1}.day^{-1}$	SC = 500; RRGS = 0.10; RRDS = 0.10; RRFI = 0.15; RRFS = 0.10; RRRF = 0.05
	RRS	Relative rate of secondary infection	0.10, 0.075, 0.05 or 0.025	plant. $plant^{-1}.day^{-1}$	

Table 3. Sensitivity analysis of white mould model performed using 144 simulated epidemics. Area under disease progress curves (AUDPC) were used to compare parameters effect. SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage

Parameters	Range values	AUDPC
SC	1000	1887
	500	1494
	100	637
	10	95
RRGSC	0.01	515
	0.05	1036
	0.10	1234
	0.15	1329
RRDS	0.05	1158
	0.10	1061
	0.15	981
	0.20	914
RRFI	0.01	427
	0.05	1175
	0.10	1585
	0.15	1820
RRFS	0.01	1633
	0.05	1411
	0.10	1167
	0.20	795
RRRF	0.05	1493
	0.10	1305
	0.15	1161
	0.20	1047
RRP	0.005	1282
	0.01	1692
	0.02	2095
	0.04	2426
RRS	0.025	1605
	0.050	1812
	0.075	1977
	0.100	2100

Table 4. Correlation matrix of white mould model parameters that were used to simulate 144 white mold (*Sclerotinia sclerotiorum*) epidemics and dimensions of principal component analysis with significance of p <0.05. (SC), relative rate of germinated sclerotia (RRGS), relative rate of death of sclerotia (RRDS), relative rate of flower infection (RRFI), relative rate of flower senescence (RRFS), relative rate of removed flowers (RRRF), relative rate of primary infection on foliage (RRP), and relative rate of secondary infection on foliage (RRS). (Incidence) proportion of diseased plants, coefficients of determination of the temporal analyses of monomolecular (MONO) and logistic models (LOGI) "adjusted" coefficient of determination of regressions described by Campbell and Madden (1990b), (AUIPC) area under incidence progress curve.

Variables	PCA1	PCA2	PCA3
SC	0.67	ns	ns
RRGSC	0.36	ns	ns
RRDSC	ns	ns	ns
RRFI	0.43	-0.18	-0.18
RRFS	-0.22	0.34	0.34
RRRF	ns	0.61	0.61
RRP	ns	ns	ns
RRS	ns	-0.82	-0.82
Incidence	0.90	-0.26	-0.26
AUIPC	0.93	-0.32	-0.32
Monomolecular Regression (MONO)	0.64	0.71	0.71
Logistic Regression (LOGI)	-0.84	-0.17	-0.17
Eigenvalue	3.67	1.91	1.39
Explained Variance (%)	30.58	15.96	11.63
Cumulative variance	30.58	46.55	58.18

Dim = dimension. ns= not significant at p<0.05.

Table 5. Summary of the parameters of 144 simulated epidemics generated with the proposed white mould model. Parameters of model: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage; AUDPC – Area under disease progress curve

Parameters								
Low Intensity Epidemics (LIG)								
	SC	RRGSC	RRDS	RRFI	RRFS	RRRF	RRP	RRS	AUDPC
N of Cases					44				
Minimum	10	0.01	0.05	0.01	0.01	0.05	0.02	0.05	16.29
Maximum	500	0.15	0.2	0.1	0.2	0.2	0.02	0.05	716.22
Median	100	0.1	0.1	0.1	0.05	0.1	0.02	0.05	226.99
Arithmetic Mean	249,09	0.076	0.12	0.066	0.076	0.114	0.02	0.05	309.53
Standard Deviation	233,85	0.044	0.046	0.043	0.058	0.038	0	0	227.70
Moderate Intensity Epidemics (MIG)								
	SC	RRGSC	RRDS	RRFI	RRFS	RRRF	RRP	RRS	AUDPC
N of Cases					86				
Minimum	100	0.01	0.05	0.01	0.01	0.05	0.005	0.025	746.41
Maximum	1000	0.5	0.2	0.15	0.2	0.2	0.02	0.1	2189.44
Median	500	0.1	0.1	0.1	0.05	0.1	0.02	0.05	1568.63
Arithmetic Mean	526.74	0.094	0.109	0.099	0.068	0.107	0.019	0.051	1505.75
Standard Deviation	216.07	0.033	0.037	0.029	0.051	0.047	0.004	0.01	440.26
High Intensity Epidemics (HIG)								
	SC	RRGSC	RRDS	RRFI	RRFS	RRRF	RRP	RRS	AUDPC
N of Cases					14				
Minimum	500	0.1	0.05	0.1	0.01	0.05	0.02	0.025	2239.44
Maximum	1000	0.15	0.15	0.15	0.05	0.1	0.04	0.1	2500.03
Median	500	0.1	0.1	0.1	0.05	0.05	0.02	0.05	2332.40
Arithmetic Mean	678.57	0.111	0.096	0.111	0.041	0.071	0.026	0.057	2341.31
Standard Deviation	248.62	0.021	0.024	0.021	0.017	0.026	0.009	0.021	86.46

Table 6. ANOVA table of groups generated from area under disease progress curve (AUDPC). The first group was characterized by low AUDPCs. The second group was characterized by intermediary AUDPCs. The third group was characterized by high AUDPCs where: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage

Source	Type III SS	df	Mean Squares	F-Value	p-Value
SC	3007576	2	1503788	29.76	0.00
Error	7123623	141	50522		
RRGSC	0.016	2	0.008	6.14	0.00
Error	0.180	141	0,001		
RRDS	0.007	2	0.004	2.41	0.09
Error	0.215	141	0.002		
RRFI	0.038	2	0.019	17.28	0.00
Error	0.157	141	0.001		
RRFS	0.012	2	0.006	2.39	0.09
Error	0,368	141	0,003		
RRRF	0.019	2	0.010	5.32	0.01
Error	0.256	141	0.002		
RRP	0.001	2	0.000	17.29	0.00
Error	0.002	141	0.000		
RRS	0.001	2	0.000	2.75	0.06
Error	0.014	141	0.000		

Table 7. Effect of white mould epidemic groups on model parameters, where: SC- Number of sclerotia on the system (5 m^2 of dry beans); RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infection on foliage; RRS- Relative rate of secondary infection on foliage

Epidemic Groups	Arithmetic Mean of Model Parameters							
	SC	RRGSC	RRDS	RRFI	RRFS	RRRF	RRP	RRS[*]
Low intensity epidemics	249.09 A	0.076 A	0.120 ns	0.066 A	0.076 ns	0.114 A	0.020 A	0.050 A
Moderate intensity epidemics	526.74 B	0.094 B	0.109 ns	0.099 B	0.068 ns	0.107 A	0.019 A	0.051 AB
High intensity epidemics	678.57 C	0.111 B	0.096 ns	0.111 B	0.041 ns	0.071 B	0.026 B	0.057 B

Means followed by same letters in the column do not differ statistically according to Tukey´s test (P≤0.05).
ns = not significant (P≤0.0 5).at 5%.
* = significance level (P≤0.06).at 6%.

Table 8. Canonical discriminant functions standardized by within variances carried out with 144 simulated bean white mould epidemics

Factors	Discriminant functions	
	F1	F2
Constant	-6.686	2.635
SC	0.988	0.252
RRGSC	0.608	0.022
RRDS	-0.384	0.006
RRFI	0.864	0.375
RRFS	-0.347	0.154
RRRF	-0.360	0.333
RRP	0.460	-0.845
RRS	0.197	-0.219

Parameters of white mould model: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infections on foliage; RRS- Relative rate of secondary infections on foliage

Table 9. Jackknifed classification matrix of discriminant analysis performed with 144 simulated bean white mould epidemics using the following model parameters: SC- Number of sclerotia on soil per 5 m^2 of dry beans; RRGS- Relative rate of germinated sclerotia; RRDS- Relative rate of death of sclerotia; RRFI- Relative rate of flower infection; RRFS- Relative rate of flower senescence; RRRF- Relative rate of removed flowers; RRP- Relative rate of primary infections on foliage; RRS- Relative rate of secondary infections on foliage

|	LIG[a]	MIG	HIG	% correct
Low Intensity Epidemic Group	42	2	0	95
Moderate Intensity Epidemic Group	13	67	6	78
High Intensity Epidemic Group	0	2	12	86
Total	55	71	18	84

[a]LIG, MIG and HIG indicate low, moderate and high intensity epidemic groups, respectively.

CONCLUSÕES GERAIS

O ciclo da doença causada por *Sclerotinia sclerotiorum* no feijoeiro-comum é bastante complexo, e pode ser dividido em quatro fases, correspondendo a estruturas típicas do ciclo de vida do patógeno. Essas fases correspondem à formação e função biológica de quatro tipos de propágulos: escleródios, apotécios e ascósporos, pétalas infectadas e hifas infecciosas. Normalmente, o mofo-branco do feijão é tratado como pertencente ao modelo de monocíclico ("juros simples", sensu Van der Plank) de epidemias. Embora a grande importância do inóculo inicial seja evidente, a fenomenologia do mofo-branco se revelou claramente mais complexa do que é geralmente descrito na literatura. As análises dos conjuntos de epidemias experimentais possibilitaram a identificação de três grupos distintos. O primeiro grupo (Grupo A) inclui epidemias de lento estabelecimento, ("tardívagas", sensu Gäumann). As epidemias do Grupo C iniciam de forma precoce, progredindo muito rapidamente com uma taxa de infecção inicialmente elevada, seguida de estabilização. As epidemias do Grupo B apresentam um comportamento intermediário entre os grupos A e C, inicialmente com baixa taxa de progresso da doença, seguido por um forte aumento da taxa de infecção da folhagem em fases posteriores. Devida à maior frequência de contatos efetivos entre patógeno e hospedeiro nas epidemias do grupo C, as infecções planta-a-planta ocorrem com frequência. Portanto, mesmo se o inóculo primário na forma de escleródios e apotécios seja, em parte, relevante, o papel das infecções subsequentes por outros propágulos como as hifas infecciosas (Propágulo 4) devem ser levados em conta. Da mesma forma, as análises realizadas com as saídas do modelo simplificado de mofo-branco do feijão permitiram identificar os principais parâmetros para o progresso da doença. As análises indicam que número de escleródios (SC), taxa relativa da germinação de escleródios (RRGS), taxa relativa da

infecção de flores (RRFI), taxa relativa de infecção primária (RRP) e taxa relativa de infecção secundária (RRS) foram os parâmetros mais importantes do modelo de simulação de epidemias de mofo-branco. Portanto, mesmo que a quantidade de inóculo inicial (na forma de propágulo 1, escleródios) permaneça como um dos mais importantes parâmetros, o patossistema feijão (*Phaseolus vulgaris*) – *S. sclerotiorum* é muito mais complexo e foge do modelo teórico de epidemia monocíclica. Portanto, epidemias que apresentam diversos propágulos efetivos (epidemias multi-propágulo), como o mofo-branco do feijoeiro, podem ser muito complexas, diferindo dos padrões monocíclicos e policíclicos tradicionalmente descritos ('juro simples' e 'juro composto' sensu Van der Plank).

ANNEXES
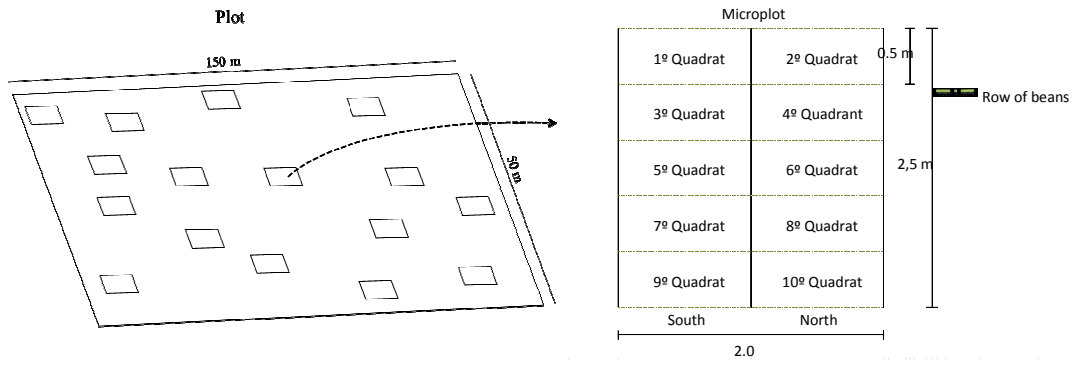
ANNEX 1. Design of plots, microplots and quadrats studied as bean white mould epidemic units.

ANNEX 2. Best fitting growth models for describing disease progress of white mould of bean in function of three epidemics groups, totalling 40 epidemics.

Group[a]	Epidemic Nº	Epidemic Code	Logistic prediction							Monomolecular prediction						
			a	SEa	b	SEb	R*²	F	P	a	SEa	b	SEb	R*²	F	P
A	1	E2AM3	0.001	0.003	0.600	0.218	0.623	7.613	0.070	0.003	0.004	0.730	0.258	0.636	7.995	0.066
A	2	E2AM4	-0.044	0.092	1.246	0.411	0.672	9.194	0.056	0.009	0.040	0.948	0.178	0.872	28.309	0.013
A	3	E2AM9	-0.032	0.140	1.180	0.568	0.453	4.318	0.129	0.024	0.043	0.883	0.173	0.863	26.139	0.014
A	4	E2AM11	-0.029	0.100	1.235	0.612	0.435	4.074	0.137	0.013	0.029	0.898	0.175	0.863	26.258	0.014
A	5	E2AM14	-0.005	0.008	1.245	0.305	0.797	16.663	0.027	0.002	0.005	0.886	0.188	0.842	22.262	0.018
A	6	E2BM2	0.001	0.003	0.600	0.218	0.623	7.613	0.070	0.003	0.004	0.730	0.258	0.636	7.995	0.066
A	7	E2BM4	-0.005	0.008	1.245	0.305	0.797	16.663	0.027	0.002	0.005	0.886	0.188	0.842	22.262	0.018
A	8	E2BM7	-0.039	0.096	1.254	0.478	0.596	6.890	0.079	0.009	0.034	0.941	0.166	0.886	32.065	0.011
A	9	E2BM8	-0.021	0.093	1.199	0.609	0.418	3.871	0.144	0.012	0.027	0.901	0.178	0.861	25.676	0.015
A	10	E2BM15	-0.026	0.107	-0.026	0.107	0.389	3.544	0.156	0.015	0.030	0.894	0.176	0.861	25.849	0.015
A	11	E2BM17	0.003	0.003	0.281	0.120	0.531	5.527	0.100	0.008	0.007	0.499	0.292	0.323	2.908	0.187
A	12	E2BM20	0.003	0.003	0.281	0.120	0.531	5.527	0.100	0.008	0.007	0.499	0.292	0.323	2.908	0.187
B	13	E2AM1	-0.063	0.119	1.131	0.349	0.704	10.535	0.048	0.008	0.067	0.971	0.197	0.854	24.398	0.016
B	14	E2AM2	-0.048	0.032	1.515	0.219	0.922	47.994	0.006	0.012	0.031	0.900	0.207	0.817	18.833	0.023
B	15	E2AM12	-0.007	0.163	0.886	0.232	0.773	14.627	0.031	-0.678	0.667	1.794	0.945	0.394	1.794	0.945

B	16	E2BM1	-0.010	0.024	1.175	0.275	0.812	18.283	0.023	0.014	0.023	0.769	0.261	0.658	8.687	0.060
B	17	E2BM5	-0.048	0.031	1.408	0.149	0.957	89.204	0.003	0.011	0.045	0.937	0.216	0.817	18.847	0.023
B	18	E2BM6	-0.041	0.024	1.493	0.167	0.952	80.271	0.003	0.009	0.029	0.922	0.197	0.839	21.819	0.019
B	19	E2BM10	-0.051	0.068	1.079	0.170	0.907	40.234	0.008	0.001	0.121	1.005	0.303	0.714	10.977	0.045
B	20	E2BM13	-0.041	0.141	1.095	0.499	0.488	4.818	0.116	0.029	0.057	0.876	0.204	0.814	18.513	0.023
B	21	E2BM14	-0.038	0.056	1.379	0.370	0.763	13.902	0.034	0.009	0.028	0.924	0.187	0.854	24.489	0.016
B	22	E2BM21	-0.022	0.132	1.069	0.612	0.339	3.052	0.179	0.034	0.048	0.809	0.221	0.756	13.370	0.035
B	23	E2BM22	-0.062	0.093	1.048	0.208	0.859	25.458	0.015	-0.015	0.128	1.042	0.288	0.751	13.092	0.036
C	24	E2AM5	0.010	0.015	0.998	0.021	0.998	2303.000	0.000	-0.811	0.811	1.957	1.133	0.332	2.984	0.183
C	25	E2AM6	0.003	0.015	0.981	0.021	0.998	2174.925	0.000	-0.830	0.890	1.989	1.280	0.262	2.417	0.218
C	26	E2AM7	-0.012	0.019	0.994	0.026	0.997	1489.091	0.000	-0.617	0.647	1.707	0.862	0.422	3.923	0.142
C	27	E2AM8	0.000	0.042	1.025	0.059	0.987	301.336	0.000	-0.900	0.815	2.101	1.154	0.367	3.314	0.166
C	28	E2AM10	0.029	0.104	1.020	0.154	0.914	43.622	0.007	-0.968	0.890	2.242	1.316	0.322	2.903	0.187
C	29	E2AM13	0.043	0.034	0.939	0.044	0.991	461.310	0.000	0.300	0.274	0.719	0.353	0.441	4.153	0.134
C	30	E2AM15	-0.068	0.069	1.075	0.094	0.970	131.838	0.001	-0.945	0.633	2.159	0.859	0.571	6.318	0.087
C	31	E2AM16	-0.068	0.069	1.075	0.094	0.970	131.838	0.001	-0.945	0.633	2.159	0.859	0.571	6.318	0.087
C	32	E2AM17	-0.005	0.127	1.051	0.185	0.887	32.361	0.011	-1.054	0.818	2.375	1.193	0.425	3.960	0.141
C	33	E2BM3	-0.037	0.048	1.361	0.299	0.832	20.774	0.020	0.008	0.031	0.932	0.193	0.848	23.289	0.017
C	34	E2BM11	-0.075	0.135	1.196	0.360	0.715	11.038	0.045	0.008	0.049	0.976	0.131	0.931	55.243	0.005
C	35	E2BM16	-0.092	0.209	1.075	0.434	0.562	6.141	0.089	0.060	0.057	0.871	0.119	0.930	53.928	0.005

|															
C	36	E2BM18	-0.046	0.153	0.963	0.349	0.624	7.639	0.070	0.029	0.098	0.929	0.224	0.802	17.233	0.025
C	37	E2BM19	-0.068	0.147	1.099	0.337	0.707	10.650	0.047	0.017	0.067	0.958	0.153	0.905	38.966	0.008
C	38	E2BM23	-0.020	0.187	-0.020	0.187	0.624	7.650	0.070	0.111	0.125	0.815	0.211	0.778	14.986	0.031
C	39	E2BM24	-0.057	0.194	1.094	0.511	0.472	4.580	0.122	0.050	0.061	0.855	0.161	0.871	28.024	0.013

[a] Groups of white mould epidemics identified with hierarchical cluster analysis. R^{*2}= Coefficients of determination for agreement between observed and predict incidenc
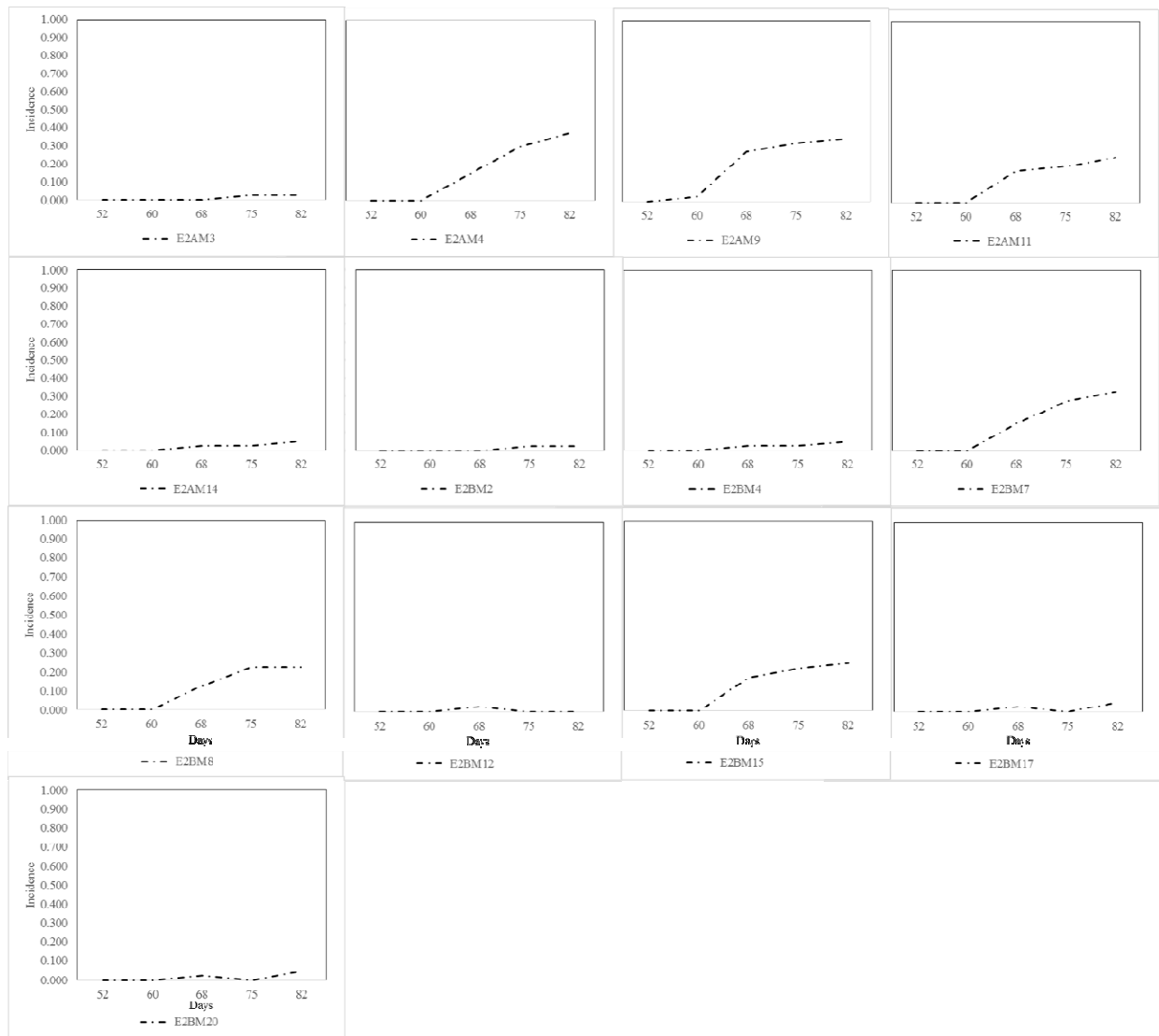
Annex 3. Disease progress curves by microplot in group A. **DAS**: Days after sowing. **E2A**: Experimental set A in 2014. **E2B**: Experimental set B in 2014. **M**: Experimental Unit, microplot.
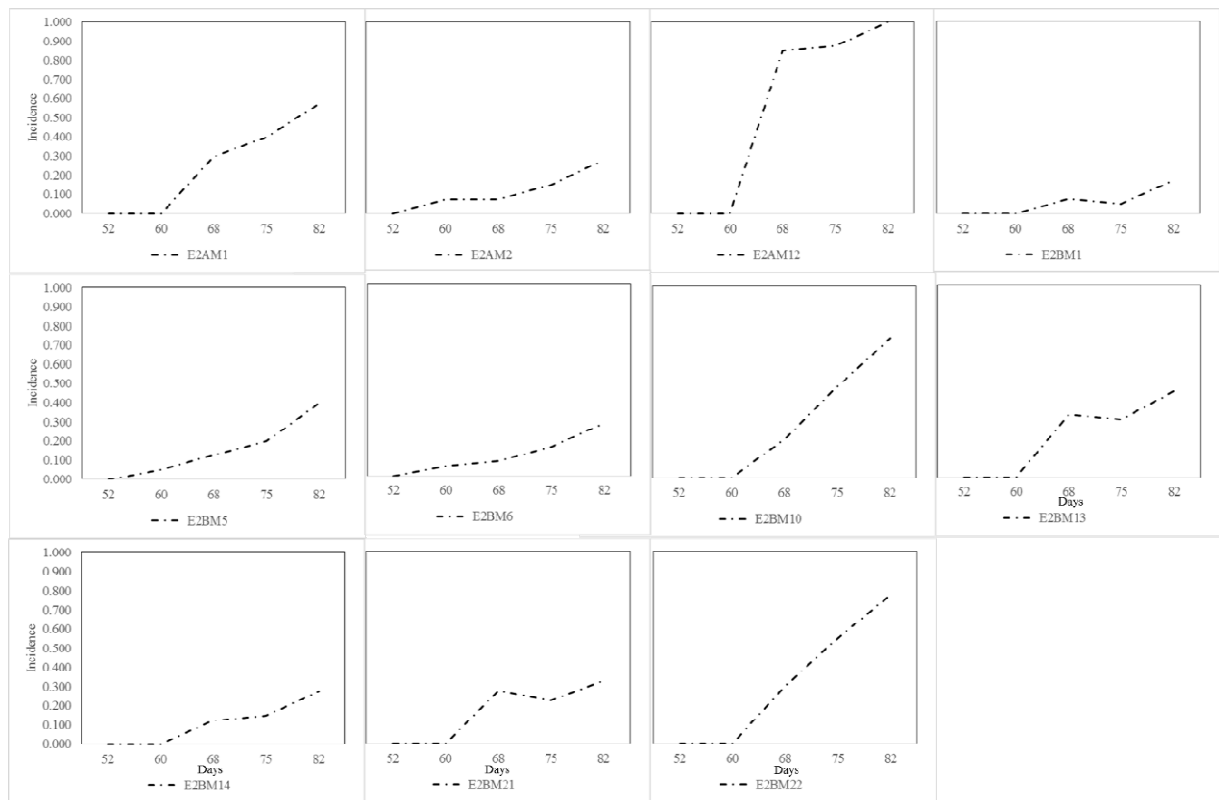
Annex 4. Disease progress curves by microplot in group B. **DAS**: Days after sowing. **E2A**: Experimental set A in 2014. **E2B**: Experimental set B in 2014. **M**: Experimental Unit, microplot.
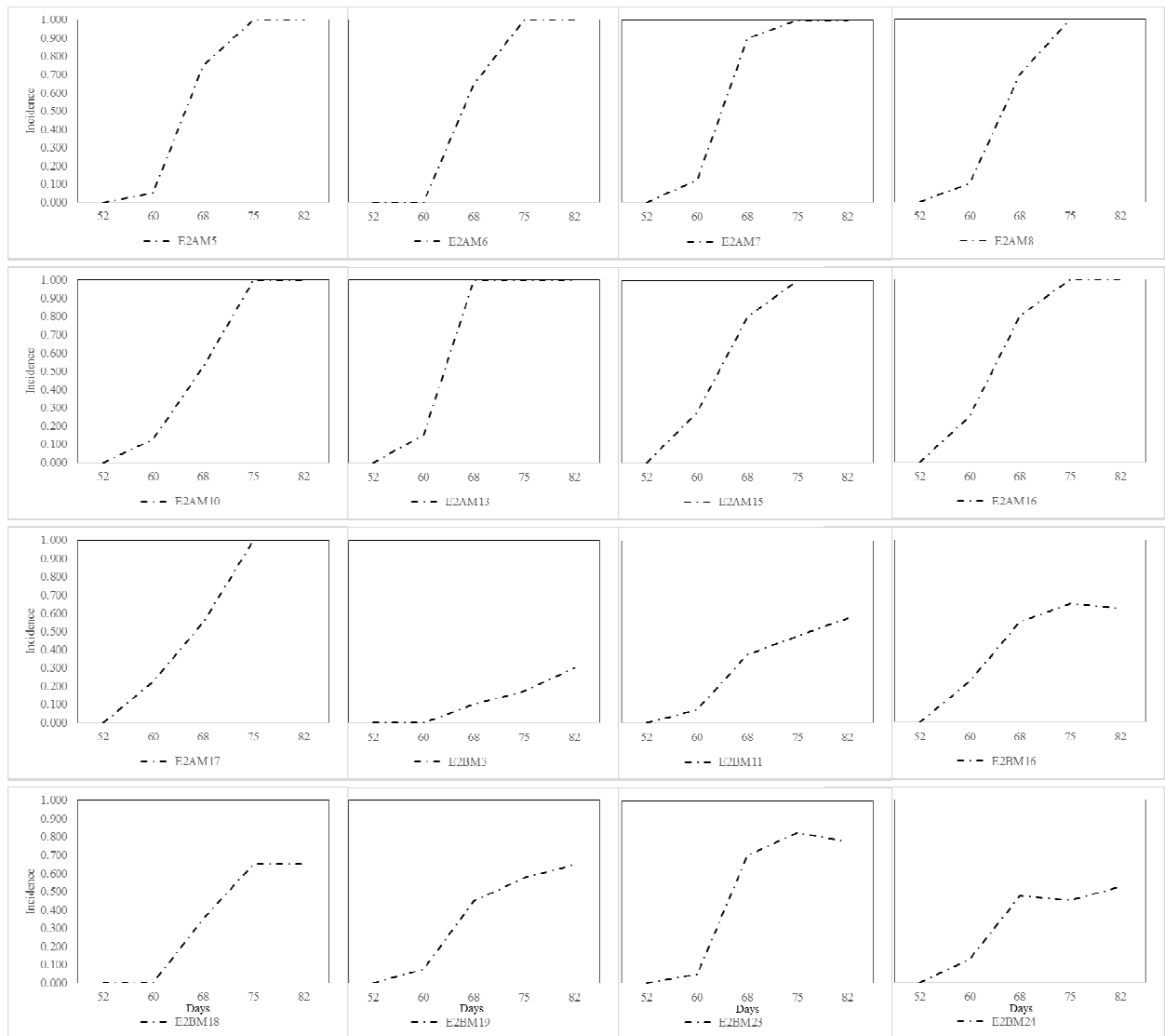
Annex 5. Disease progress curves by microplot in group C. **DAS**: Days after sowing. **E2A**: Experimental set A in 2014. **E2B**: Experimental set B in 2014. **M**: Experimental Unit, microplot.