

Annemarie Pielaat

Splash

**The dispersal of fungal plant
pathogens in rain events**

Urr 921307

Promotor: Dr. M.J. Jeger
Hoogleraar Ecologische fytopathologie

Co-promotor: Dr. F. van den Bosch
Universitair hoofddocent,
Leerstoelgroep wiskundige methoden en modellen

PN08701, 2785.

Annemarie Pielaat

Splash

The dispersal of fungal plant pathogens in rain events

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Dr. C.M. Karssen,
in het openbaar te verdedigen
op vrijdag 12 mei 2000
des namiddags te 13.30 uur in de Aula.

1512 975804

Pielaat, A., 2000

Splash, the dispersal of fungal plant pathogens in rain events.

PhD Thesis Wageningen University, Wageningen, the Netherlands.

-With ref.- With summary in Dutch.

ISBN 90-5808-212-1

Subject headings: Splash / dispersal / *Pyrenopeziza brassicae* / *Colletotrichum acutatum*
/ modelling

Voorwoord

Het is dinsdagavond 15 december 1999, zowel deze eeuw als het werk loopt tot een eind; laat ik daarom maar eens beginnen. Toen ik in de zomer van 1995 bij de vakgroep wiskunde op sollicitatiegesprek kwam was mijn eerste indruk; houten deuren, een balustrade en nog veel meer houten deuren. De maandag daarop ging de telefoon; „met Frank van den Bosch; zelf stond je er nogal sceptisch tegenover, maar ik denk dat je het wel kunt”. Het lijkt er op dat je gelijk had, toch, wanneer je dacht dat het de verkeerde kant op ging stond je bij me op de stoep, niet te vaak hoor, zo'n 5 keer per week. Dat heeft er wel toe geleid dat dit proefschrift nu gewoon af is. Frank, ik wil je bedanken voor je creativiteit en inzet gedurende deze vier jaar, wat ertoe geleid heeft dat dit proefschrift nu in deze vorm voor me ligt.

I would also like to thank my promotor Mike Jeger for his insight in epidemiological processes and his insight in the relevance of our research to knowledge on the spatial spread of splash dispersed plant pathogens. Our discussions helped me to formulate the right research questions and how to obtain relevant answers. I wished we could have had more of these discussions. I am grateful to Larry Madden, Bruce Fitt and Alastair McCartney for a successful cooperation and for making my papers interesting to Phytopathologists. Op mijn eerste werkdag werd ik voorgesteld aan Angelique Lamour. Toen zij tijdens de lunch haar broodtrommel(“tje”) openmaakte en daaruit een bolus tevoorschijn haalde was het ijs gebroken. Angelique, ik wil je bedanken voor zo'n 7200 minuten zwemtraining wat ons een totale afstand van zo'n 360 km had kunnen opleveren als we niet zoveel te bespreken hadden gehad. Je staat altijd voor een ander klaar en ik ben dan ook heel blij dat je mijn paranimf wil zijn.

Na een jaar werd mijn leven als enige oio bij de vakgroep abrupt beëindigd toen Mark Huiskes mijn kamergenoot werd. Mark, ik heb je leren kennen als iemand met het beste gevoel voor humor, want voor minder dan de overtreffende trap ga je niet. Toch ben je altijd kritisch, erg nieuwsgierig, je kunt heerlijk zeuren en je hebt me altijd geholpen met allerlei computer- en wiskunde problemen. Kortom, ik had me geen betere kamergenoot kunnen wensen.

Een half jaar daarna mochten we Eric Boer verwelkomen. Eric, ik benijd je om je rustige, evenwichtige persoonlijkheid en weloverwogen uitspraken waarmee je me altijd weer met beide benen op de grond wist te zetten. Gelukkig vind je het ook leuk grapjes met onze aio-collega's uit te halen, maar zeg je „nee” als je het genoeg vindt, want je hebt principes of je hebt ze niet. Onze “Coppa d'amore” zal ik niet snel vergeten.

Maria-João Paulo en Anna Rzepiela made our team complete. João, dank je voor je Nederlandse gezelligheid, je opgewekte karakter en de vele glaasjes melk die je voor

me mee gebracht hebt wanneer ik niet mee ging lunchen. Anna, thank you for being a sensible unprejudiced person. You could convince me to stop working, and do something nice instead, with so little effort.

Langzamerhand ben ik ook tot de ontdekking gekomen dat er achter ieder van die houten deuren op onze vakgroep een andere persoonlijkheid schuil gaat. Ik heb het als een voorrecht ervaren in zo'n diverse groep te mogen werken waarin iedereen altijd bereid was te helpen waar nodig. In het bijzonder wil ik Maarten de Gee bedanken voor zijn inzet in de letterlijk en figuurlijk donkere dagen voor kerst. Zonder jouw fysisch inzicht had dit proefschrift geen zesde hoofdstuk gehad. Onno van Herwaarden wil ik bedanken voor alle hulp bij het voorbereiden van mijn onderwijstaak en de bemoedigende woorden in de laatste periode van dit onderzoek. Bij Johan Grasman wil ik graag nog eens een cursus tijdsmanagement volgen. Je was er wanneer nodig, handelde officiële zaken het liefst dezelfde dag nog af en stipt om twaalf uur op maandag stond je ook nog eens klaar om te gaan zwemmen.

Nathalie van der Wal wil ik bedanken voor haar onvermoeibare inzet toen ons onderzoek keer op keer weer vastliep. Jouw enthousiasme en rationele kijk op het leven hebben mij zeker geïnspireerd tijdens dit onderzoek en daarbuiten. Doug Marshall, thank you for passing so many lonely hours in the rain tower. Believe it or not, these have been very useful hours, resulting in a more thorough understanding of the wonderful splash world.

Mijn vrienden Jeannette, Frank, Esther, Marna, Frank, Petra, Orson, Loes en Willem wil ik bedanken voor hun jarenlange trouw en onvergetelijke terugkomdagen waarin zowel klassieke als luxe problemen tot in de details doorgrond werden.

Gerrit, mijn man, aan jou wil ik dit proefschrift opdragen, want misschien had ik in een andere werkomgeving ook een baan als onderzoeker in opleiding kunnen hebben, maar ik weet zeker dat dit proefschrift er nu niet lag als jij er niet altijd voor me was geweest. Gerrit, ik wil je bedanken voor je steun de afgelopen jaren, want mezelf kennende weet ik wat een zware taak dat is geweest.

Tot slot wil ik mijn ouders bedanken dat ze mij een opvoeding hebben gegeven waarbij doorzetten een belangrijke rol heeft gespeeld. Ik hoop met dit boekje aangetoond te hebben waar zo'n opvoeding allemaal niet toe kan leiden.

Annemarie Pielaat

Abstract

Models were developed to study splash dispersal of fungal plant pathogens in space and time. The models incorporate the main mechanisms involved in splash dispersal, that is *i.* A raindrop hits the thin water film on the crop surface containing spores and spores are dispersed in the splashing rain droplets, and *ii.* Splashed spores are redistributed in the crop and on the soil surface. A mechanistic random 'jump' model describes the stochastic processes of splash dispersal over a homogeneous surface from a point source. Numerical analysis showed the importance of ground cover and rain intensity as factors determining model output. More spores were splashed in high intensity rains and, simultaneously, more spores were removed from the system. A diffusion approximation was developed for this mechanistic model which could only be considered a reasonable approximation under certain limiting conditions. Based on the two-dimensional version of the mechanistic model an equation was developed for the total number of spores in the area surrounding an inoculum source over time, $N(t)$. In addition, equations for the expected mean, $E(r)$, and mean squared distance, $E(r^2)$, spores travel during a rain event at a given time were developed. Observed data and model predictions showed that both $N(t)$ and $E(r^2)$ increased to a maximum over time and then declined due to spore removal from the system and depletion of spores at the source. Factors influencing the process could be assessed by changing parameter values.

Upward displacement of lesions by stem extension and dispersal of fungal conidia by rain-splash are mechanisms contributing to within-crop disease spread. These mechanisms were incorporated into a model based on the interaction between winter oilseed rape and the light leaf spot pathogen (*Pyrenopeziza brassicae*) as an example. Experimental results showed that most conidia were dispersed during a 15 min duration of rainfall. The trajectory of a droplet depended on the impacted plant part, with a mean horizontal travel distance decreasing with increasing incident drop diameter and a maximum splash height which ranged from 0.3 cm when splashed from a flower up to 57 cm for a pod. These results were incorporated into the model. Stem extension was shown to be an important factor influencing vertical disease spread. Rain events contributed to the splash dispersal of conidia to the plant apex and resulting lesions were directed vertically by internode growth. Periods with frequent rain events in a dense crop canopy were most favorable for disease progress. The upward spread of light leaf spot on winter oilseed rape in experiments at the Institute of Arable Crops Research, Harpenden, UK, was similar to that predicted by the model. Finally, an analytical model was proposed to study the influence of crop characteristics and rain properties on the vertical spread of splashed spores. Splash dispersal was concentrated in the upper layers in a crop having a constant or increasing leaf surface area with height. The greatest splash probabilities occurred and most spores were intercepted in the layers just below the apex of a crop having a decreasing leaf surface area with height.

Contents

1	Introduction	1
2	A model for dispersal of plant pathogens by rain-splash	5
3	Spores splashing under different environmental conditions: a modelling approach	25
4	Trajectories of splash droplets during splash of <i>Pyrenopeziza brassicae</i> (light leaf spot) conidia from oilseed rape plant parts	51
5	Simulation of vertical spread of plant diseases in a crop canopy by stem extension and splash dispersal	63
6	A theory on the vertical dispersal of splashy units influenced by crop characteristics	87
7	General conclusions	103
	References	105
	Samenvatting	112
	Curriculum vitae	116

Chapter 1

Introduction

Splash is an important biophysical mechanism by which many thousands of infectious pathogen units, such as fungal spores, are spread within a crop in short periods of time (Fitt *et al.*, 1989; Madden, 1992). This form of dispersal is a component of the epidemic cycle for many serious above-ground fungal diseases of crops in temperate and tropical climates, but is often not recognized as the second most important mechanism of spread for plant pathogens (Madden, 1992). Symptoms of fungal diseases are often manifested as lesions on the surface of leaves and other tissues. As soon as rain starts infectious units (spores) of the lesion are released into the water layer formed on the leaf surface (Figure 1.1a). If a raindrop hits this water layer it will break into thousands of splash droplets. Spores are incorporated into these droplets and are dispersed to other sites in the canopy (Figure 1.1b). The initial splash when a raindrop strikes infected plant material is characterized as the primary splash (Fitt *et al.*, 1989). At its new site, the spore can either initiate a new infection or again be incorporated in a splash droplet and travel further in the crop by secondary splashes. The distance a spore can travel from its original site depends not only on the rain intensity and duration, but also on characteristics of the spore, the ground cover and the canopy structure (Fitt *et al.*, 1992; Madden *et al.*, 1993; Yang and Madden, 1993). Many pathogens have both airborne (often sexual) spores and splashborne (often asexual) spores. Disease epidemics are often initiated by airborne ascospores produced on plant debris remaining on the field after the crop is harvested and transported into a new crop in autumn. Subsequent gradual horizontal and vertical disease spread is often by splash dispersed conidiospores (Inman *et al.*, 1999).

Experimental work on the dissemination of splash dispersed pathogens has intensified considerably in recent years. A rain simulator can be used to study the dispersal process, and effects of rain, canopy, ground, and pathogen properties can be assessed (Fitt *et al.*, 1986; Madden *et al.*, 1996; Reynolds *et al.*, 1987; Yang *et al.*, 1991). Many data sets

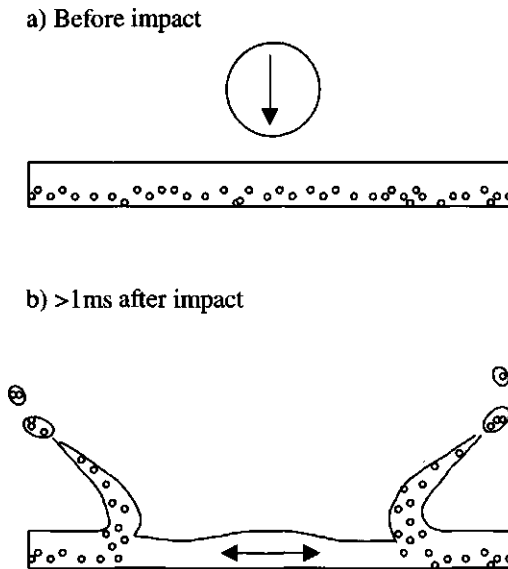


Figure 1.1: Splash; the process of spore dispersal in splash droplets as raindrops strike thin films of water covering spores (Fitt *et al.*, 1989). When the surface tissue underneath the water film consists of a sporulating lesion the process is characterized as primary splash (Chapters 4 and 5). When spores in the water film are splashed from healthy surface tissue the process is characterized as secondary splash (Chapters 2, 3, 4, 5 and 6).

are available on the spatial spread of diseases such as anthracnose and leather rot on strawberry fruit and on the spread of light leaf spot and white leaf spot on winter oilseed rape. Other important diseases with splash dispersed infectious units are eyespot, septoria leaf blotch and glume blotch on wheat; leaf blotch on winter barley; ear blight and scab on cereals; white tip disease on leek; stem canker on soybean; leaf spot on tomatoes; and brown rot, canker and scab on apples. Nevertheless, splash dispersal in different plant-pathogen systems, and the influence of both biological and environmental factors on disease spread cannot be quantified through experimental research alone. There is a need for physical models in which the mechanisms involved in splash dispersal are incorporated through, e.g. probability functions. Firstly there is a probability per unit time that a raindrop hits a site in the crop or on the ground at which the surface water contains spores, secondly a probability that a spore will be dispersed in the splashing rain droplets, and finally there is a probability density function for the spatial spread of splashed droplets. With these models the spatial spread of spores in different plant-

pathogen systems can be studied, because factors influencing the splash process are incorporated as separate parameters in the probability functions of the models. The values of these parameters can easily be changed for different conditions. However, mechanistic models for predicting realistic patterns of disease spread require estimates of parameter values for the separate processes obtained from experiments. Once reasonable parameter values are incorporated in the model the most important factors influencing disease spread can be investigated. This can guide further experimental research. Based on this philosophy of linking theoretical models with an experimental programme, several mechanistic models have been developed in the research described in this thesis to investigate the spatial spread of rain splashed spores. Experiments were performed and published data sets were used to provide reasonable parameter values for the models. The models were then tested with laboratory and field data from different plant-pathogen systems.

The influence of ground cover and rain intensity on spore travel distances when splashed from a point-source has been studied in many experiments (Madden *et al.*, 1996; Yang *et al.*, 1990). Few models have however been developed that describe splash dispersal in general, independent of biological and environmental conditions. Our first intention was therefore to qualify the dispersal process with a mechanistic model. For this purpose a one-dimensional mechanistic random jump model is introduced in Chapter 2. This model qualifies the dispersal process of spores from a point source over a flat surface on the ground as measured during experiments. Since many spatial processes are successfully described by a diffusion equation, the model is compared to a previously published diffusion model by making a diffusion approximation for the mechanistic model. Whereas the original diffusion model consisted of composite parameters, it was now possible to partition these parameters as part of the underlying biological processes. In addition, it is shown under what conditions the diffusion model is a valid approximation to the full model.

Of course, spores jump in two dimensions when a flat surface is considered and this approach is needed to quantify splash dispersal. The two-dimensional version of the model for horizontal spread is discussed in Chapter 3. Determining the distances spores travel has been a goal of much experimental work. Two biologically interesting measures are derived from the model in this respect. Firstly an equation for the change in total number of spores in the area surrounding the source from the start of the rain event is derived. Secondly equations for the mean and mean-squared distance displacement of spores from the original point-source are determined. The model is tested by comparing theoretical results with previously published experimental results on the dispersal of conidia from an infected strawberry source fruit in relation to surface topography and rain intensity.

Not only horizontal, but also vertical spread of disease to the upper leaves and seed-bearing organs is an important mechanism causing damaging epidemics in winter-sown arable crops in Europe. The development of, for example, pods in oilseed rape and

ears in cereals determines the yield of these crops. Epidemics are normally initiated in the autumn and winter and subsequently upward spread is by stem extension and rain-splash in spring (Inman and Fitt, 1992; Inman *et al.*, 1999). However, the relative importance of splash dispersal and upward spread of infected tissue by stem extension is still unclear. Insight into the main mechanisms involved in the upward spread of plant diseases can guide predictions on disease severity under different biological and environmental conditions. Therefore, a generic simulation model for vertical disease spread is developed. Light leaf spot on winter oilseed rape is used as a model system. Experiments were performed to obtain reasonable parameter values for the physical processes in this model and these are described in Chapter 4. The percentage of spores being splashed from a leaf was assessed for different drop size impactions and rain durations in a rain simulator. This resulted in knowledge on primary and secondary splash processes for this plant-pathogen system under different conditions. In addition, the "average" ballistic trajectories of splash droplets were estimated from experiments in which a single raindrop impacted on a particular plant part or the ground and the travel distance of splash droplets was determined. This resulted in insight into spore travel distances when splashed from different surfaces during rain events.

In Chapter 5 the full simulation model is developed, including a deterministic plant growth approach and splash parameters obtained from the experiments. During rain events spores are splashed to the plant apex where new plant parts develop and are directed to the upper canopy by internode growth. Vertical spread of light leaf spot during the growth of oilseed rape is investigated for different conditions concerning disease initiation and rain duration. Because of its general structure, the model is applicable for different plant-pathogen systems.

Simulation results showed that crop structure, in addition to other biological and environmental factors, is an important factor influencing disease spread. The influence of leaf surface area distribution in a crop on the vertical spread of splashed spores is studied further in Chapter 6. An analytical model is proposed and the influence of crop characteristics and rain properties on vertical spread is investigated by specifying three different crop idiotypes and varying rain parameters.

The main conclusions of this research are given in Chapter 7.

Stellingen

1. Door de geleidelijke afname van sporen aan de bron kan een diffusiemodel geen nauwkeurige beschrijving geven van de initiële spatverspreiding
(Dit proefschrift)
2. Frequente regenbuien in het voorjaar kunnen veel schade aanrichten in een gewas. Een regenbui veroorzaakt voornamelijk horizontale spatverspreiding van sporen naar de in deze periode uitgroeiende plantdelen. Met een daaropvolgende stengelstrekking worden lesies vertikaal in het gewas getransporteerd van waaruit, door horizontale spatverspreiding, weer nieuwe plantdelen kunnen worden geïnfecteerd.
(Dit proefschrift)
3. Over dit proefschrift:
"Se non è vero, è ben trovato"; Als het niet waar is, is het aardig gevonden.
4. Communicatie met wetenschappers is als een computertaal; één term verkeerd en ze geven een foutmelding.
5. Toevallig de beste zijn is moeilijker dan toevallig de slechtste zijn.
6. De status van man en vrouw wordt pas gelijk als mannen zich bij het huwelijk bereid tonen de naam van de vrouw aan te nemen.
7. Dat weinig vrouwen hoge posities bekleden hebben zij aan niemand anders dan aan zichzelf te wijten, dan wel te danken.
8. Opname van het "pimpampoentje" in de "van Dale" toont aan dat Zeeuws-Vlamingen in de spraakmakende gemeente beginnen door te dringen. Het is een kwestie van tijd of ik kan gewoon weer om een puntmesje vragen of een foto trekken zonder dat iemand er gek van opkijkt.

9. Carpoolstroken zijn alleen dan nuttig wanneer er weinig gebruik van wordt gemaakt.
(L.A., 1997)

10. Stijging van de welvaart gaat jammergenoeg gepaard met een daling van de tolerantie.

11. Zeeuws-Vlamingen, een dubbel-zijn, Zeeuws in hun nuchterheid en Vlaams in hun gastvrijheid.

A. Pielaat

Splash -The dispersal of fungal plant pathogens in rain events-
Wageningen, 12 mei 2000

Chapter 2

A model for dispersal of plant pathogens by rain-splash

A. Pielaat F. van den Bosch¹

Abstract

A mechanistic random 'jump' model is developed to describe the stochastic processes of splash dispersal of plant pathogens from a point-source. In this model the main physical processes involved in the spatial spread of these spores are incorporated. That is, the probability per unit time that a spore is splashed λ , the probability that it then travels over some distance $D(x)$, and the probability that it is not removed during this dispersal process ε . Numerical analysis shows the importance of ground cover and rain intensity on the model output. Factors influencing the process can be captured by changing the parameter values. For high rain intensities λ is large, therefore more spores are splashed; and, since ε is expressed per splash, simultaneously more spores will be removed from the system. The effect of ground cover is captured by ε ; its value decreases if the probability of staying in the process decreases. In addition, an equation is derived for the mean squared distance that spores splash. This equation shows a linear function independent of $D(x)$. Finally, a diffusion approximation is developed for the mechanistic model and is compared to a diffusion model for splash dispersal developed by Yang *et al.* (1991). The diffusion equation can only be considered a reasonable approximation to the full model under certain limiting conditions.

¹IMA Journal of Mathematics Applied in Medicine and Biology (1998) 15, 117-134.

2.1 Introduction

Dispersal of spores by rain-splash droplets is, next to wind, the second most important dispersal mechanism for above-ground fungal plant pathogens (Madden, 1992).

Prior to the dispersal event spores will usually be embedded in the plant surface. There they are sheltered from movement until it starts raining. During the rain event, spores will readily be suspended in the water layer formed on the plant surface. When a raindrop hits the water layer it will, depending on its kinetic energy and the surface tension, cause the water layer to break up into many splash droplets. Spores can be incorporated into these splash droplets and deposited again at different positions from the original point of impact (Fitt and McCartney, 1985).

This phenomenon of disease dispersal was first demonstrated at the beginning of this century by Stepanov and, subsequently, by Gregory (1961). In the 1980s Fitt and associates were the first to experimentally investigate the dispersal of spores from single-drop impactions. Since then, further developments in experimental techniques have allowed detailed measurements of the factors affecting dispersal of spores from a point source by rain-splash (Fitt *et al.*, 1989; Reynolds *et al.*, 1987; Yang *et al.*, 1990; Yang and Madden, 1993). Most of this research has been performed under laboratory conditions. A rain simulator has been used to mimic real rain events. In these experiments raindrops with different size distributions can be produced to impact on an infected source (Reynolds *et al.*, 1987). This source can consist of just a concentrated number of spores on the ground up to an infected plant canopy. Changing the raindrop properties (for example mass and impact velocity), ground cover, inoculum source or canopy structure allows the influence of these factors on spore dispersal to be determined. Although many data sets are available, few models have been developed to allow insight in the spread of these infectious units. The majority are descriptive models which do not include the mechanisms involved in splash dispersal. Increases in knowledge of these mechanisms facilitates the development of models which describe splash dispersal, in general, independently of crop-specific properties. Such models will increase the reliability of the prediction of further disease spread by splash dispersal.

Yang *et al.* (1990) measured splash dispersal from infected strawberry fruits with a rain simulation system. Yang *et al.* (1991) used a diffusion equation to estimate parameters describing the overall process of spore dispersal in space and time. The one-dimensional version of the model they used is

$$y = \frac{Q}{2\sqrt{\pi\alpha t}} e^{-\frac{r^2}{4\alpha t}} e^{-\beta t}, \quad (2.1)$$

where $y=y(r,t)$ represents the number of spores per unit distance at a position r from the source at time t during a rain event, Q is the number of spores available for dispersal at $t=0$, α is a parameter related to the coefficient of dispersal, reflecting the spores' random

motion over distance per unit time, and β is the constant of spore loss from the system (time^{-1}). Because of their simplicity, diffusion models are frequently used to describe biological systems. However, parameters in model (2.1) do not describe the separate mechanisms steering dissemination of fungal plant pathogens by rain splash-droplets. This is particularly true for the coefficient of dispersal, which represents a process that depends on several factors such as the probability per unit time of being splashed and the distribution of the splash distances. But how, for example, can this diffusion coefficient α be calculated from experiments on the rate of spore dispersal and experiments on the distance a spore is splashed? Further, spore loss β is expressed as a constant per unit time. However, spore loss is related to splash events. It is not immediately clear how β is related to the probability that a spore is lost from the process when it is splashed.

The purpose of this paper, therefore, is to develop a mechanistic model for the spatial spread of splash dispersed infectious units. Our first intention is to qualify the dispersal process of spores from a point source over a flat surface on the ground as measured during experiments. Modelling the redistribution of spores during a rain event requires a stochastic description. Solving the model results in a function which gives the probability of finding a spore at any single distance from the source during a rain event. A numerical study is performed to get insight into the sensitivity of model output to different parameter values.

Since many spatial processes are successfully described by a diffusion equation, we also derive a diffusion approximation to the mechanistic model. For this purpose redistribution of spores in a rain is first described with a random walk model (Edelstein-Keshet, 1988). The diffusion model is compared to the mechanistic model. We also compare the model to the above mentioned diffusion model of Yang *et al.* (1991). This leads to a subdivision of the composite parameters into the separate basic mechanisms underlying splash dispersal.

2.2 The model

The stochastic displacement of spores during a rain event is described by a random 'jump' model. Considering the experimental set-up, initially spores splash from a point source and are subsequently displaced over a flat ground surface. To quantify the spatial spread of spores this process would best be described in two dimensions. However, since our main interest is in assessing the qualitative aspects of spatial spread, a one-dimensional model is appropriate as a starting point. The output of this one-dimensional model is completely analogous to the two-dimensional case except for quantitative differences. To develop the model we first discuss the major mechanisms involved in splash dispersal.

As soon as it starts raining, plant pathogen spores are suspended in the water layer that forms on the leaf surface. If a raindrop hits this water layer, a spore can remain in place or be incorporated into one of the droplets formed and be spread. The probability

per unit time of a spore being splashed during a rain event is denoted by λ . Madden *et al.* (1996) showed how λ can be estimated from experiments. In these experiments infected strawberry fruits with known spore density are placed on the ground. A rain simulator is used to measure spore removal at different rain intensities (in millimetres per hour). To quantify the proportion of spores that are splash dispersed, the remaining spore density was determined after a particular rain application time. The range of values for λ was used to indicate a reasonable parameter value to use as the input for the model. In natural systems the value of λ is influenced not only by rain properties but also by aspects of the canopy structure, such as leaf angle and surface roughness. So λ can be set to an other default value that depends on these factors which affect the system.

When a spore is splashed it is redeposited at a distant position. To determine the spatial distribution of spores during a rain event, results from experiments on frequency distributions of droplet travel distances can be used. With a videographic system, Yang *et al.* (1991^a) determined droplet travel distances. For the model we define $D(x-\xi)$ as the probability density function (p.d.f.) for a spore to jump from position ξ , to position x once it is splashed. It is assumed that there is no canopy nor wind so the p.d.f. for dispersal from ξ is radially symmetric.

During a rain event, spores can be removed from the system. Each time a spore is splashed it has a certain probability of being washed off into the soil by raindrops or of being deposited at a place where further dispersal is not possible. This indicates that a spore has a probability per splash of leaving the process which means that it is excluded from any further spread. Therefore, the probability of a spore staying in the process per splash is introduced as ε . Although ε cannot be measured directly during experiments, ground cover was found to be one of the main factors influencing this parameter (Madden *et al.*, 1993). When straw rather than plastic was used as ground cover, there was less disease incidence. In natural systems ε captures more than the effect of ground cover alone. Spores can, for example, also get stuck to the underside of a leaf which prevents it from being splashed again. Therefore, a range of ε is considered to show the qualitative effect on model output.

A model for splash dispersal during rain events can be developed on the basis of these mechanisms. The number of spores at position x from the source at time t during a rain event is expressed as $N(t,x)$. The rate of change in the number of spores at position x at time t equals the rate at which spores are splashed away from position x and the rate at which spores are deposited at position x . So,

$$\frac{\partial N(t,x)}{\partial t} = -S_1(t,x) + S_2(t,x), \quad (2.2)$$

where $S_1(t,x)$ is the number of spores splashed per unit time from x and $S_2(t,x)$ is the number deposited at x per unit time. The probability per unit time of a spore being

splashed away is λ ; therefore,

$$S_1(t, x) = \lambda N(t, x). \quad (2.3)$$

Now $S_2(t, x)$ depends on the probability being deposited at x when a spore is splashed from any other position, say ξ , and the number of spores present at ξ . The probability of travelling some distance has been defined by the p.d.f. $D(x - \xi)$. Taking into account the probability that a spore is actually splashed away and not removed from the process during its journey, we thus have

$$K(t, x, \xi) = \varepsilon \lambda N(t, \xi) D(x - \xi). \quad (2.4)$$

where $K(t, x, \xi)$ represents the number of spores that leave ξ and are redeposited at x . To calculate $S_2(t, x)$ one has to integrate over all possible places. Therefore,

$$S_2(t, x) = \int_{-\infty}^{\infty} K(t, x, \xi) d\xi. \quad (2.5)$$

Using the scaling

$$P(t, x) = \frac{N(t, x)}{\int_{-\infty}^{\infty} N(0, x) dx}, \quad (2.6)$$

we finally find

$$\frac{\partial P(t, x)}{\partial t} = -\lambda P(t, x) + \int_{-\infty}^{\infty} \varepsilon \lambda P(t, \xi) D(x - \xi) d\xi, \quad (2.7)$$

where $P(t, x)$ can be interpreted as the proportion of the total number of spores present at the start of the rain event, deposited at a particular position x after some period of rain. Note that $P(t, x)$ also represents the probability per unit length of finding a spore at position x at time t .

To assess the distances that spores can travel from their initial point of impact, experiments were performed by, for example, Huber *et al.* (1997) and Reynolds *et al.* (1989). These experiments usually started with one point source on a wet target surface. Rainfall was then generated during which spores were splash dispersed. For the model such a point source can be introduced as a Dirac delta function; that is, at the start of the rain event, $t=0$, all the spores lie at one source, indicated by $x=0$. In this case the model has an initial condition given by $P(0, x) = \delta(x)$.

2.2.1 Solving the model equation

Solving the model equation will result in the probability function of a spore being found at position x at time t during a rain event. Equation (2.7) can be solved using the bilateral Laplace transformation

$$\mathcal{L}\{f(x)\} = \int_{-\infty}^{\infty} e^{-sx} f(x) dx. \quad (2.8)$$

For a discussion on integral transforms readers can refer to a book by Sneddon (1972). If the differential equation is transformed with respect to position, it follows that

$$\frac{\partial \mathcal{L}\{P(t, s)\}}{\partial t} = (-\lambda + \varepsilon \lambda \mathcal{L}\{D(s)\}) \mathcal{L}\{P(t, s)\}, \quad (2.9)$$

in which $\mathcal{L}\{P(t, s)\}$ and $\mathcal{L}\{D(s)\}$ are the transforms of $P(t, x)$ and $D(x)$, respectively. Solving equation (2.9) yields

$$\mathcal{L}\{P(t, s)\} = C e^{(-\lambda + \varepsilon \lambda \mathcal{L}\{D(s)\})t}. \quad (2.10)$$

Given the initial condition, which states that at the start of the rain event all spores still lie at the source, that is $P(0, x) = \delta(x)$, it follows that $\mathcal{L}\{P(0, s)\} = 1$ and therefore that $C=1$. Applying the inverse transformation \mathcal{L}^{-1} leads to

$$P(t, x) = e^{-\lambda t} \mathcal{L}^{-1}\{e^{\varepsilon \lambda \tilde{D}(s)t}\}. \quad (2.11)$$

Expanding the exponential term in a Taylor series around $t=0$ one obtains

$$P(t, x) = e^{-\lambda t} \mathcal{L}^{-1}\left\{\sum_{i=0}^{\infty} \frac{(\varepsilon \lambda \tilde{D}(s)t)^i}{i!}\right\}, \quad (2.12)$$

and the solution of (2.7) is

$$P(t, x) = e^{-\lambda t} \delta(x) + \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i D(x)^{*i}. \quad (2.13)$$

This solution has a straightforward interpretation. The term $e^{-\lambda t} \delta(x)$ indicates that particles at the source decrease exponentially in number. With this term we can calculate

the fraction of spores, at any time during the rain event, that has still not been splashed from the source. The summation on the right-hand side represents the spatial spread once a spore is actually splashed from its point of impact; the quantity $(\lambda t)^i e^{-\lambda t} / i!$ is the Poisson distribution, which gives the probability that a spore is splashed i times at some time during the rain event; ϵ^i gives the probability that this spore is not removed from the system each time it is splashed; $D(x)$ gives the p.d.f. for a spore travelling to position x once it is splash dispersed one time from the source. Then the probability distribution for the position a spore can take if it is splash dispersed twice is given by

$$D(x)^{*2} = \int_{-\infty}^{\infty} D(\xi) D(x - \xi) d\xi. \quad (2.14)$$

In this equation $D(x - \xi)$ gives the p.d.f. for a spore to jump from ξ to x given the probability that in the previous jump it travelled to position ξ , indicated by $D(\xi)$. During its first journey, a spore can be splash dispersed to many different places from the source. Integrating over all possible places from which a spore then can be splashed the second time gives $D(x)^{*2}$. Of course, spores can be splashed many times during a rain event, all having the same p.d.f. for spatial redistribution. So, to incorporate the spatial distribution of spores that are splashed i times during a rain event one has to use the repeated convolution $D(x)^{*i}$ defined as

$$D(x)^{*i} = \int_{-\infty}^{\infty} D^{*i-1}(\xi) d(x - \xi) d\xi \quad \text{for } (i = 2, 3, 4, \dots). \quad (2.15)$$

Adding the distributions of the spores for all possible times they can be splashed during some period of rain (that is, summation over i) gives $P(t, x)$.

2.2.2 Analysis of the solution

In this section we study the solution of the mechanistic model (2.13). First the p.d.f. for spatial distribution of spores during a rain event is determined. Then a numerical study is performed to obtain insight into the sensitivity of model output for different parameters.

In experiments where $D(x)$ is measured the number of spores decrease monotonically with distance from the source (Yang *et al.*, 1991^a). This trend can be approximated by the normal distribution

$$D(x) = \frac{1}{\sqrt{2\pi}\sigma} \cdot e^{-\frac{x^2}{2\sigma^2}}, \quad (2.16)$$

and the double-exponential distribution

$$D(x) = (\sigma\sqrt{2})^{-1} e^{-|x|\frac{\sqrt{2}}{\sigma}}. \quad (2.17)$$

In both p.d.f.s $\text{Var}(x) = \sigma^2$.

The convolution, $D(x)^{*i}$ of the normal distribution is

$$D(x)^{*i} = \frac{1}{\sqrt{2\pi i\sigma}} \cdot e^{\frac{-x^2}{2i\sigma^2}} \quad (2.18)$$

(Mood *et al.*, 1950), and for the double-exponential distribution

$$D(x)^{*i} = \frac{1}{\phi} \cdot \frac{e^{\frac{-|x|}{\phi}}}{2^{2i-1}(i-1)!} \sum_{j=1}^{i-1} \frac{2^j(2i-j-2)!}{j!(i-j-1)!} \left|\frac{x}{\phi}\right|^j, \quad (2.19)$$

in which $\phi = \frac{\sigma}{\sqrt{2}}$ (Johnson and Kotz, 1970).

Using the solution to (2.7), in combination with these distributions for $D(x)^{*i}$, the spread of infectious units in relation to the source is studied. Therefore, all figures represent the summation on the right-hand side of (2.13) without the initial source. The results given below will show how the use of different distributions for $D(x)^{*i}$ affects dispersal. The main purpose is to determine the sensitivity of $P(t, x)$ to different model parameters after the resulting dissemination, both in space and time. We mentioned earlier in Section 2 how λ and ε can be estimated from experiments. Experimental results were considered to give an indication of the range in which reasonable parameter values can be found. Values for λ were found to vary between 0.02 min^{-1} (with a rain intensity of 4 mm h^{-1}) and 0.06 min^{-1} (with a rain intensity of 60 mm h^{-1}). After considering of both the values measured and the rain intensities used the default parameter value was set to $\lambda = 0.05 \text{ min}^{-1}$.

Parameter values for ε can be calculated by combining experimental results given by Yang *et al.* (1991), who measured β and Madden *et al.* (1996), who measured λ . In both these experiments, parameter values were measured for different rain intensities and ground covers. Using the equation $\beta = \lambda(1 - \varepsilon)$ (a derivation of this equation is discussed in section 3) ε values for ε can be calculated. The mean values calculated for ε were: 0.6 splash^{-1} for plastic, 0.5 splash^{-1} for soil and 0.2 splash^{-1} for straw. However, these mean values were calculated from widely varying parameter values and rain intensities. Therefore, we assume a reasonable parameter value for ε is 0.7 splash^{-1} .

The third parameter is σ , which occurs in both (2.18) and (2.19). Yang *et al.* (1991^a) determined spore dispersal from single-drop impactions. The distances that spores traveled varied between 0 and 20 cm. However, most spores traveled in the range 2-7cm.

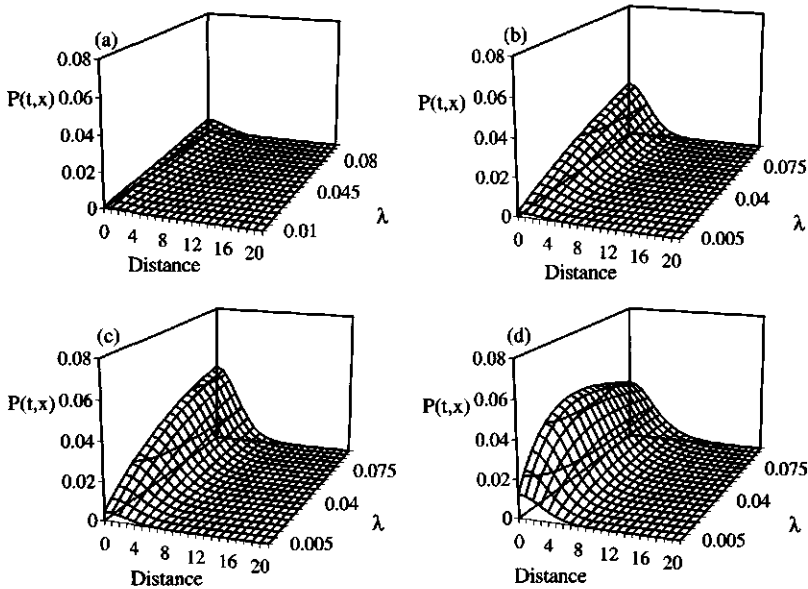


Figure 2.1: The effect of λ , the probability of being splashed per unit time, on $P(t, x)$, the probability per unit length of finding a spore at position x (cm) during rain for four times: (a) $t=1$, (b) $t=5$, (c) $t=10$, and (d) $t=30$. (When a normal p.d.f. is used for $D(x)^{*i}$, the spatial spread of spores, the probability of not being removed from the process, $\varepsilon=0.7$; and the standard deviation for $D(x)^{*i}$ is $\sigma=3$.)

Considering this frequency distribution of droplet travel distances, a default value of 3 cm was used for σ . With these parameter values the following results were obtained. Extra terms were added to the summation in (2.13) until the contribution of the next term to the total sum was less than 0.001%; that is, the summation was from $i=1$ to $i=99$.

In Figures 2.1 and 2.2 the probability of a spore travelling some distance during a rain event was plotted for varying values of λ . Figures 2.1 and 2.2 show the results when the normal distribution and the double-exponential distribution are used, respectively, for $D(x)$. Figures 2.1 and 2.2 show that shortly after the start of the rain event (that is, up to $t=10$) the majority of the spores are still concentrated near the source. As λ increases, the spores have a larger probability of being splashed from the source per unit of time, and therefore the probability of being in the process also increases. But, since it has only been raining for 10 minutes, even spores with the largest values of λ could not travel very far from the source. For $t=30$ the curve shows a maximum at least up to

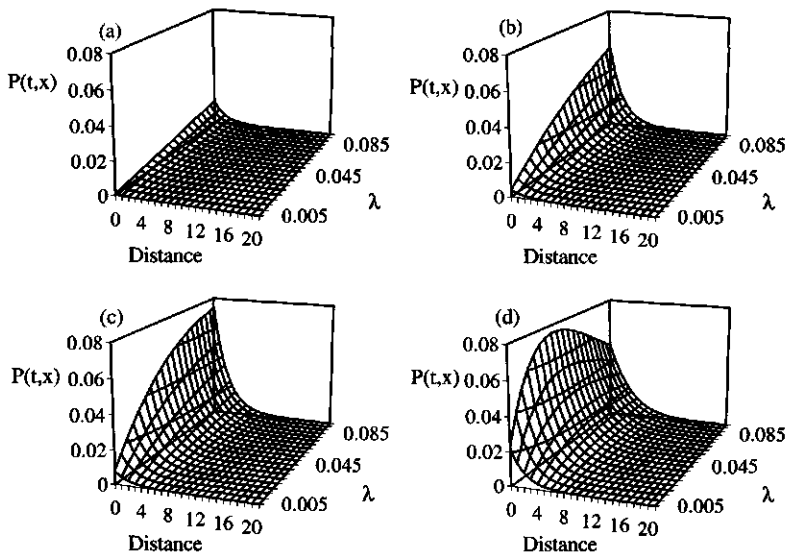


Figure 2.2: The effect of λ , the probability of being splashed per unit time, on $P(t, x)$, the probability per unit length of finding a spore at position x (cm) during rain for four times: (a) $t=1$, (b) $t=5$, (c) $t=10$, and (d) $t=30$. (When a double-exponential p.d.f. is used for $D(x)^{*i}$, the spatial spread of spores, the probability of not being removed from the process, $\varepsilon=0.7$; and the standard deviation for $D(x)^{*i}$ is $\sigma=3$.)

6 cm, as λ increases. This could be caused by ε , the probability of staying in the process per splash.

As the probability of being splashed (λ) increases, more spores will be removed from the process if it continues raining. However, Figures 2.3 and 2.4 show that, at least up to 50 minutes of rain, the probability of travelling any distance increases. Therefore ε has no influence on this trend. This effect is caused by depletion of spores near the source. Since λ is expressed per unit of time, spores will already have passed short distances from their initial point of impact at $t=30$. Figures 2.3 and 2.4 show the dependence of the model solutions on the variable ε , illustrating the results when the normal distribution and the double-exponential distribution, respectively, are used for $D(x)$. If the probability of staying in the process increases, the probability of being dispersed some given distance will increase for at least 50 minutes. After almost two hours of rain λ influences the process in addition to ε . At $t=100$, even spores with $\lambda=0.05$ per time unit are likely to have passed short distances from their source; therefore, the curve is decreasing as $t \geq 100$. Also, spores with a small probability of staying in the process per splash will have already disappeared after 100 min of rainfall, and so the probability of travelling

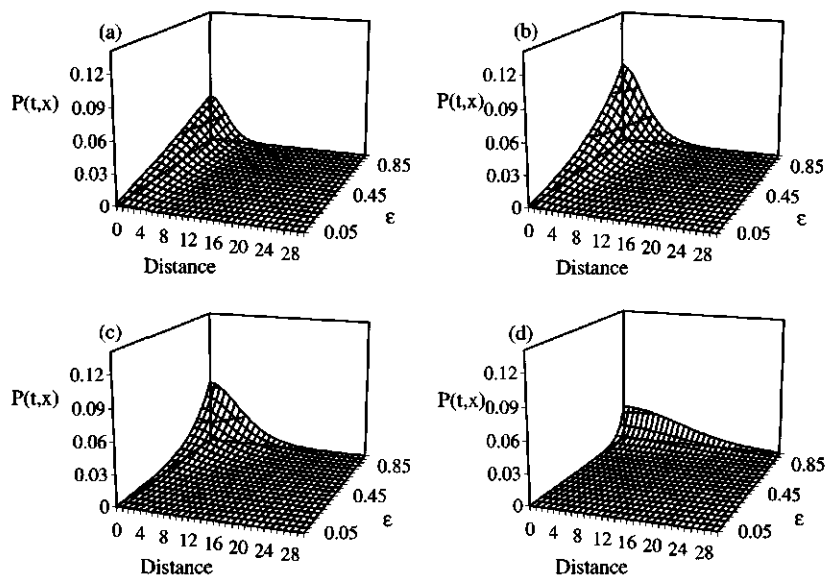


Figure 2.3: The effect of ϵ , the probability of not being removed from the process per splash event, on $P(t, x)$, the probability per unit length of finding a spore at position x (cm) during rain for four times: (a) $t=10$, (b) $t=50$, (c) $t=100$, and (d) $t=300$. (When a normal p.d.f. is used for $D(x)^{*i}$, the spatial spread of spores, the probability of being splashed per unit time is $\lambda=0.05$; and the standard deviation for $D(x)^{*i}$, $\sigma=3$).

any distance decreases for values of ϵ smaller than, say, 0.55. Yet, spores with larger values of ϵ will have had sufficient time to travel short distances from the source without being removed from the process. Therefore, the probability of reaching a relatively large distance from the source increases if ϵ is sufficiently large.

When comparing Figure 2.1 with 2.2 and Figure 2.3 with 2.4, with their different p.d.f.s, it can be seen that the figures are almost identical. They only differ for small rain application times.

2.2.3 The mean squared displacement

One of the main goals in experimental research on splash dispersal is to assess the distance dispersed during a rain event. Determining the mean squared distance that spores travel from the source is the most frequently used method for statistical analysis of resulting data sets (Othmer *et al.*, 1988). In addition to its practical advantages, that

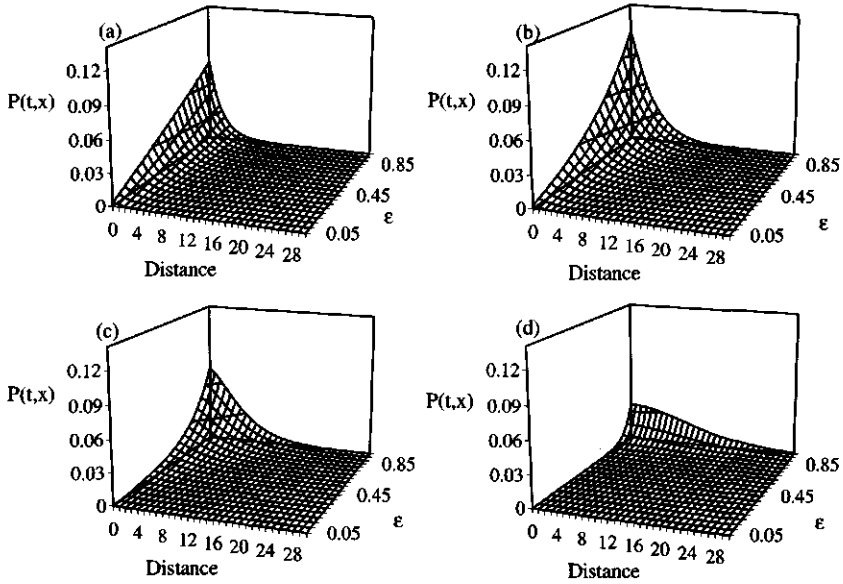


Figure 2.4: The effect of ϵ , the probability of not being removed from the process per splash event, on $P(t, x)$, the probability per unit length of finding a spore at position x (cm) during rain for four times: (a) $t=10$, (b) $t=50$, (c) $t=100$, and (d) $t=300$. (When a double-exponential p.d.f. is used for $D(x)^{**}$, the spatial spread of spores, the probability of being splashed per unit time, $\lambda=0.05$, and the standard deviation for $D(x)^{**}$, $\sigma=3$.)

is, it is relatively easy to calculate the mean squared distance of spores, this method also gives a good impression of the surface over which spores are dispersed round the source. Moreover, both a one-dimension and a two-dimensions derivation results in the same equation.

Since the expectation, $E(x)$, equals zero, the mean squared displacement is the variance which is defined as

$$Var(x) = \int_{-\infty}^{\infty} x^2 P(t, x) dx,$$

where $P(t, x)$ is the probability function for spatial distribution of spores, then

$$Var(x) = \int_{-\infty}^{\infty} x^2 e^{-\lambda t} \delta(x) dx + \int_{-\infty}^{\infty} x^2 \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i D(x)^{*i} dx.$$

The first term on the right-hand side vanishes because the variance for spores at the source is 0. Therefore, we only have to account for the second term, which can be written as

$$Var(x) = \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i \int_{-\infty}^{\infty} x^2 D(x)^{*i} dx.$$

Since the variance of a sum of independent random variables is the sum of the variances (Mood *et al.*, 1950), we find

$$Var(x) = \sum_{i=1}^{\infty} \frac{(\varepsilon \lambda t)^i e^{-\lambda t}}{i!} i \sigma^2,$$

where $\sigma^2 = \int_{-\infty}^{\infty} x^2 D(x) dx$ is the variance of $D(x)$. This can be simplified to

$$\begin{aligned} Var(x) &= (\varepsilon \lambda t) e^{-\lambda t} \sigma^2 \sum_{i=0}^{\infty} \frac{(\varepsilon \lambda t)^i}{i!} \\ &= \varepsilon \lambda t \sigma^2 e^{-\lambda(1-\varepsilon)t}. \end{aligned}$$

This means that for biologically realistic parameter values of ε , λ and σ the mean squared dispersed distance of spores reaches a maximum during a rain event. If it continues raining more spores will be removed from the process, and therefore the mean squared displacement decreases again.

2.3 The diffusion approximation

In modelling biological systems, diffusion equations are often used to describe random movement in space. With regard to splash dispersal, Yang *et al.* (1991) used a diffusion equation to model the spread of plant pathogens. Their justification for using a diffusion model is the assumed random motion of spores. Yet, the mechanisms behind splash dispersal indicate random jumps of spores which include the probability of being splashed per unit of time, a probability of being removed per splash and a redistribution kernel $D(x)$. As noted in Section 1, Yang *et al.* (1991) did not include the basic mechanisms

involved in splash dispersal when constructing the model. Therefore, parameter values cannot directly be calculated from experimental results. Whereas the main goal of modelling splash dispersal is to find out which physical processes involved in dispersal are of primary importance, it is interesting to establish the relation between our mechanistic model and Yang's diffusion equation.

The random-jump model (2.7) will for $t \rightarrow \infty$ and at an appropriately large spatial scale, be accurately described by a diffusion process. The diffusion model can therefore be seen as an approximation of the full mechanistic model. A relevant question is: Under what conditions is the diffusion model a valid approximation of the full model? To answer this question a diffusion approximation was derived to the mechanistic model by following the procedure outlined in Othmer *et al.* (1988) and Edelstein-Keshet (1988). For this purpose, assume that there is an equal probability of being splashed either to the left or to the right during a rain event. When, after a certain period of rain, a spore lies at some distance (say $x=\xi$) from the source ($x=0$), it has a probability of $\frac{1}{2}$ of being splashed to the left (over a distance of $-\Delta$) and to the right (over distance, $+\Delta$). For the diffusion approximation we therefore use

$$D(x - \xi) = \frac{1}{2}[\delta(x - \xi - \Delta) + \delta(x - \xi + \Delta)]. \quad (2.20)$$

Substitution in (2.7) gives

$$\frac{\partial P(t, x)}{\partial t} = -\lambda P(t, x) + \frac{\varepsilon \lambda}{2} P(t, x - \Delta) + \frac{\varepsilon \lambda}{2} P(t, x + \Delta). \quad (2.21)$$

Substituting Taylor-series expansions for $P(t, x - \Delta)$ and $P(t, x + \Delta)$ in (2.21) yields

$$\begin{aligned} \frac{\partial P(t, x)}{\partial t} = & -\lambda P + \frac{\varepsilon \lambda}{2} \left(P - \frac{\partial P}{\partial x} \Delta + \frac{1}{2} \frac{\partial^2 P}{\partial x^2} \Delta^2 - \frac{1}{3!} \frac{\partial^3 P}{\partial x^3} \Delta^3 + \dots \right) \\ & + \frac{\varepsilon \lambda}{2} \left(P + \frac{\partial P}{\partial x} \Delta + \frac{1}{2} \frac{\partial^2 P}{\partial x^2} \Delta^2 + \frac{1}{3!} \frac{\partial^3 P}{\partial x^3} \Delta^3 + \dots \right), \end{aligned} \quad (2.22)$$

where $P=P(t, x)$.

Now consider the limit of $\Delta \downarrow 0$ and $\lambda \uparrow \infty$ such that $\lambda \Delta^2$ remains constant. This means that the spores are splashed at high rates but that the distance dispersed per splash is small, so that the average distance a spore moves per unit time remains constant. However, since $\lambda \uparrow \infty$ we also have to assume that $\varepsilon \rightarrow 1$ such that $\lambda(1 - \varepsilon)$ remains constant. Only under these conditions does the diffusion equation make a sensible estimate for the mechanistic model of (2.7). It is easily seen that in this limit (2.22) becomes

$$\frac{\partial P(t, x)}{\partial t} = -\lambda(1 - \varepsilon)P(t, x) + \frac{\varepsilon\lambda\Delta^2}{2} \frac{\partial^2 P(t, x)}{\partial x^2}, \quad (2.23)$$

with initial condition $P(0, x) = \delta(x)$ (Edelstein-Keshet, 1988).

To compare the diffusion approximation to the full model, Δ^2 in (2.23) should be expressed in terms of the standard deviation σ of the dispersal kernel $D(x)$; that is,

$$\begin{aligned} \sigma^2 &= \int_{-\infty}^{\infty} x^2 D(x) dx \\ &= \int_{-\infty}^{\infty} x^2 \frac{1}{2} (\delta(x - \xi - \Delta) + \delta(x - \xi + \Delta)) dx \\ &= \Delta^2. \end{aligned} \quad (2.24)$$

Solving (2.23) (Carslaw and Jaeger, 1959) leads to

$$P(t, x) = \frac{1}{2\sqrt{\pi Dt}} e^{-\frac{x^2}{4Dt}} e^{-\lambda(1-\varepsilon)t}, \quad (2.25)$$

in which $D = \frac{\varepsilon\lambda\sigma^2}{2}$.

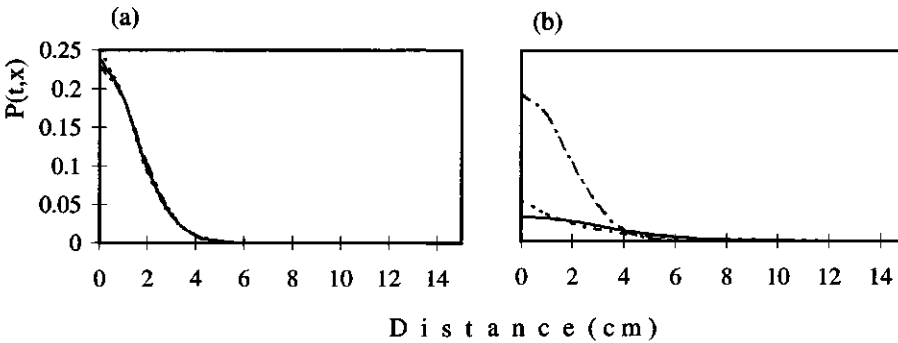


Figure 2.5: The Mechanistic model and its diffusion approximation (dashed line) for $P(t, x)$, the probability per unit length of finding a spore at position x during rain for two times: (a) $t=1$, and (b) $t=10$ (When a normal (solid line) and double-exponential (dotted line) p.d.f. are used for $D(x)^{**}$, the spatial spread of spores, the probability of being splashed per unit time is $\lambda=10$; the probability of not being removed from the process is $\varepsilon=0.99$; and the standard deviation for $D(x)^{**}$ is $\sigma=0.5$.)

Comparing this result with the model of Yang *et al.* (1991), it is now possible to partition the composite parameters as part of the underlying biological processes. The dispersal coefficient α includes several of the biological parameters introduced above, since $\alpha = \varepsilon \lambda \sigma^2 / 2$. Similarly the constant of spore loss $\beta = \lambda(1 - \varepsilon)$. Note that this parameter combination has the interpretation of the rate at which spores are removed from the process, which is similar to the interpretation of β in Yang's diffusion model.

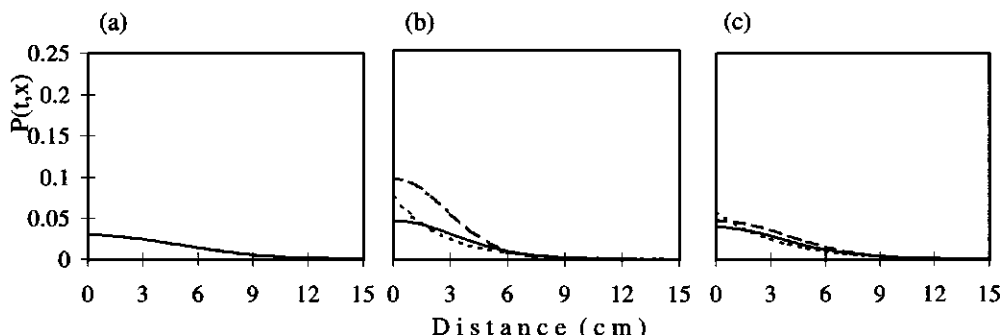


Figure 2.6: The Mechanistic model and its diffusion approximation (dashed line) for $P(t,x)$, the probability per unit length of finding a spore at position x during rain at three times: (a) $t=10$, (b) $t=25$, and (c) $t=50$. (When a normal (solid line) and double-exponential (dotted line) p.d.f. are used for $D(x)^{**}$ with $\lambda=0.05$, $\varepsilon=0.7$ and $\sigma=3$.)

In Figure 2.5 the diffusion approximation for $\lambda=10$, $\varepsilon=0.99$, and $\sigma=0.5$ is plotted together with the mechanistic model. Figure 2.5 shows the probability of a spore having position x at 1 and 10 minutes of rain. It is evident that, under these limiting conditions, the diffusion equation is indeed a sensible approximation of the mechanistic model. However, these parameter values are far from being biologically reasonable. By comparing the full model with the diffusion approximation, and using reasonable parameter values, we obtained Figure 2.6. Here the probability of a spore being splash dispersed over some distance is plotted for 10, 25 and 50 minutes of rain. At least up to 25 minutes of rain, Figure 2.6 shows that the behaviour of the diffusion equation is quantitatively different to that of the full model. That is, the diffusion model consistently overestimates densities near the source. The difference in model behaviour between the full model and its diffusion approximation can be attributed to the dynamics of the spores at the initial inoculum source. From (2.13) we see that the fraction of the spores remaining at the initial source, after a rain application of duration t , decreases exponentially as $e^{-\lambda t}$. This implies that for the parameter values used in Figure 2.6 and after 10 minutes of rain application, approximately 60% of the spores have still not been splash dispersed and remain at the initial inoculum source. After 25 minutes approximately 30%, and

after 50 minutes approximately 8% of the spores are still at the initial source. In order to derive the diffusion approximation we assumed $\lambda \uparrow \infty$, implying that the whole initial inoculum source is dispersed instantaneously at $t=0$. This explains the differences in the area under the curves in Figure 2.6. Nevertheless, in time ($t=50$) more spores will be dispersed from the source, and gradually the solutions of the full model will take the form of its diffusion approximation. However, it should be stated that these observations only hold for short distances from the source; but these short distances are of major importance for splash dispersal.

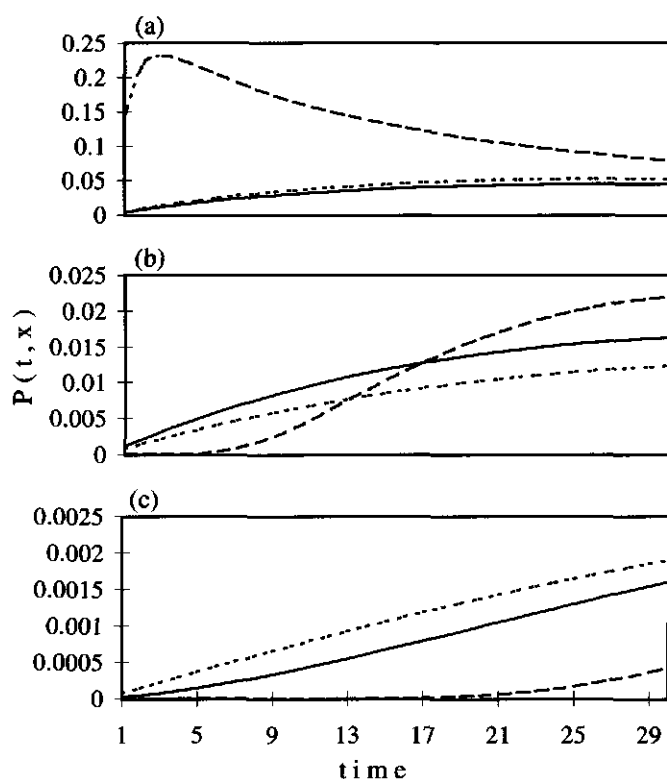


Figure 2.7: The difference between the effect of diffusion (dashed line) and mechanistic model on $P(t, x)$, the probability per unit length of finding a spore at position x at time t during rain for three distances: (a) 1 cm, (b) 5 cm, and (c) 10 cm. (When a normal (solid line) and double-exponential (dotted line) p.d.f. is used for $D(x)^{**}$, the spatial spread of spores, the probability of being splashed per unit time is $\lambda=0.05$; the probability of not being removed from the process, $\varepsilon=0.7$; and the standard deviation for $D(x)^{**}$ is $\sigma=3$.)

Figure 2.7 shows the probability of a spore, in both models, travelling either 1 cm, 5 cm or 10 cm during a rain event. Figure 2.7 shows that, for reasonable parameter values, there is not only a quantitative difference but also a qualitative difference between the full model and its diffusion equation. The original model shows an increasing probability of covering the three distances during a rain event at least up to 10 cm from the source, whereas at 1 cm from the source, the diffusion approximation first shows an increasing curve with time and then it decreases. This is caused by spatial spread of all spores as soon as $t > 0$ when applying a diffusion model. This trend continues at 5 and 10 cm; only the spores took somewhat longer time to travel these distances. Therefore, as time increases the curve shifts to the right for these distances.

In addition, note that for Figures 2.5- 2.7 the difference between using a normal p.d.f. or a double exponential p.d.f. for $D(x)$ is rather small.

Based on the above discussion, we conclude that, for reasonable parameter values and reasonable rain application times, the diffusion model is not a valid approximation of the mechanistic model. Even though quantitative differences could be smaller if a two-dimensional approach is used to compare the models, the qualitative differences will not change.

2.4 Discussion

The main goal of this study was to develop and analyse a mechanistic model for splash dispersal of plant pathogens from a point source. The most important mechanisms involved in spore dispersal by rain-splash are; the probability that a spore is splashed from its point of impact, the probability that it then travels over an arbitrary distance, and the probability that it is not removed during this dispersal process. In our model these main physical processes are represented, respectively by λ , $D(x)$, and ε . Experimental results showed that this dispersal process is influenced by many factors. However, the effect of most of these factors can be seen in their effects on the model parameters.

First, results from experiments showed the importance of ground cover on spatial spread of spores. In our model, ε includes this effect of ground cover; its value decreases if the probability of staying in the process decreases. Secondly, rainfall properties influence splash dispersal. More spores were found to be splashed, and also more spores were removed from the process during experiments with increasing rain intensities. If in the model the parameter value for λ is large, more spores will be splashed; and since ε is expressed per splash, simultaneously more spores will also be removed from the system. Finally, the probability of travelling a distance x during a splash event is also influenced by raindrop properties. For example, larger droplets can incorporate more spores but will not travel as far as small droplets. In our model, the spatial spread of spores during a splash event is represented by $D(x)$. This p.d.f. includes the parameter σ , the value of which changes according to the mean dispersal distance within one splash event.

Based on the above discussion, we conclude that the most important mechanisms for splash dispersal are incorporated in the model. In addition, many factors influencing the splash process are accounted for through their effect on the parameters. Nevertheless, the model can still be improved on several points. For example, rainfall properties can cause an additional effect. If rain intensities become large it causes spores to be washed off directly into the ground. This form of spore loss could be taken into account by making ϵ a function of λ . In addition, in natural systems the probability of being splashed from the initial source is different from the probability of being splashed once a spore is in the field. Therefore, Madden *et al.* (1996) differentiated primary splash, in which spores are removed from infected source fruit, from secondary splash, in which spores are washed off from healthy fruit. In this model we only consider secondary splash. A second point concerns the normal and double-exponential distributions, which are used to describe $D(x)$. As a first approximation this seems to be a reasonable choice since splash dispersal is often successfully described using these distributions. In addition, when analysing the model, application of these two p.d.f.s results in similar spore dispersal behaviour. However, for the mechanistic model the p.d.f. for spatial spread of spores should correspond to experimental data on frequency distributions of droplet travel distances from single-drop impactions. In addition, the probability of a spore travelling some distance should be compensated for by droplet size distribution in a rain event. In a real rain event there is a distribution of impacting drops and subsequently in splashing droplets. Since the number of spores and the dispersed distance depends on droplet size this droplet size distribution should be taken into account for the mechanistic model. Note that we are now modelling spores splashing on a horizontal surface. In natural systems the plant canopy will have an additional effect on distances that spores splash. In a subsequent study we will describe the mechanisms involved with spatial spread of spores in more detail.

The diffusion approximation for the mechanistic model showed that for splash dispersal a diffusion model is in most situations not a useful approximation. The main problem in using a diffusion model for splash dispersal is that the initial inoculum source is depleted instantaneously at $t=0$. For reasonable values of the parameter λ , however, the fraction of spores still in the initial inoculum source is decreased to 10% only after 20 to 50 minutes of rain, leading to the large differences between the mechanistic model and the diffusion equation. Of course, if it continues raining all the spores will ultimately be splashed from the source and be dispersed over relatively large distances from their initial point. In this limit for $t \rightarrow \infty$ the diffusion model describes the process as well as the mechanistic model. Still, we would like to emphasize that during each rain event spores are dispersed over relatively short distances with rain durations of up to 60 minutes maximum; this process is best described by the mechanistic approach.

Although a two-dimensional diffusion approximation possibly reduces the differences between theoretical values and observed values we want to emphasize that it will not

change the quality of the model output seen in Figures 2.6 and 2.7.

Finally, a still unanswered question is: How does the proposed mechanistic model perform if it is compared to experimental data on spore dispersal from a point source? Experimental verification is necessary to test the model and we will return to this question in a subsequent paper. Then a two-dimensional model description will be used, because testing the model involves a quantitative approach. To test the model on factors influencing the dispersal process, we will use data sets including different rain intensities as well as various ground covers.

Acknowledgements

The investigations for project nr 805-39-181 were supported by the life sciences foundation (SLW) which is subsidized by the Netherlands organization for scientific research (NWO). This article is based on a paper read at the Seventh IMA Conference on the Mathematics of Biological Systems, held in Oxford 8-10 July 1996.

Chapter 3

Spores splashing under different environmental conditions: a modelling approach.

A. Pielaat L.V. Madden G.Gort¹

Abstract

A mechanistic model was developed for the dispersal of plant pathogens by rain-splash over a homogeneous surface from a point source. The model represents number of spores per unit area and time as a function of distance r and time t , $N(t,r)$, and includes: *i.* A probability per unit time of a spore being splashed, *ii.* A probability per splash event of being removed from the dispersal process, and *iii.* A probability function for the distance a spore travels during a single splash event. Based on the model an equation is developed for the total number of spores in the area surrounding inoculum source over time, $N(t)$. Equations for the expected mean distance $E(r)$, and mean squared distance $E(r^2)$ spores travel during a rain event at a given time are also derived from the model. It is shown how the latter is used for characterizing and comparing dispersal and assessing the magnitude of disease spread. Although the model for $N(t,r)$ is complex, equations for $N(t)$ and $E(r^2)$ are relatively simple expressions of the parameters and

¹Phytopathology (1998) 88, 1131-1140.

time. The model was tested by comparing theoretical results to previously published experimental results for the dispersal of *Colletotrichum acutatum* conidia obtained using a rain simulator. In these experiments the point source consisted of infected strawberry fruit, with sporulating lesions, and the entire area received generated rain for up to 46 min. Spore deposition at selected times and distances was determined and related to experimental factors of rain intensity and surface topography. Comparing model and data indicated that the model was applicable to assess splash dispersal for a range of conditions. For example, observed data and model predictions showed that both $N(t)$ and $E(r^2)$ increased to a maximum over time and then declined, probably due to spore removal from the system and depletion of spores at the source. Factors influencing the process could be assessed by changing parameter values.

3.1 Introduction

Spores of many fungal plant pathogens are dispersed in rain-splash droplets and consequently cause new infections within a crop (Fitt and McCartney, 1985; Madden, 1992). Many of the most serious fungal diseases of crops in temperate and tropical climates are caused by pathogens with splash dispersed spores (Fitt *et al.*, 1989).

Experimental work on dissemination of splash dispersed pathogens has increased considerably in recent years (Fitt *et al.*, 1986^a; Madden *et al.*, 1996; Reynolds *et al.*, 1987; Yang *et al.*, 1991). A rain simulator often is used to study the dispersal process, and the effects of rain, canopy, surface, and pathogen properties are assessed (Fitt *et al.*, 1992; Madden *et al.*, 1993; Yang and Madden, 1993). For these experiments, an infected plant unit (e.g. fruit) is used as a point source, and the entire area receives a simulated rain. Resulting data sets on spore deposition contain information about the spatial spread from this point source for different environmental (e.g. rain intensity) and biological (e.g. crop density) conditions (Madden *et al.*, 1996; Yang and Madden, 1993). Up to now, these data sets have been used for the development of some descriptive models (Fitt and McCartney, 1986^a; Fitt *et al.*, 1987). These models, however, only contain information on the dispersal gradients for the original experiment and cannot be used to predict spatial spread for different conditions. Therefore, a model was developed that incorporates the main physical mechanisms behind splash dispersal. Factors influencing the splashing process are incorporated in parameters underlying the spatial spread of spores from a point source. The model includes: *i.* A probability per unit time of a spore being splashed, *ii.* A probability per splash of being removed from the process, and *iii.* A probability function for the distance a spore travels during a splash event. Since these are the main physical processes during splash dispersal influenced by environmental and biological conditions, their parameter values can be adapted for different circumstances.

In this paper the main mechanisms behind splash dispersal are discussed and incorporated as separate terms in the model. In addition, it is shown how the total number

of spores in the area surrounding the inoculum source changes from the start of the rain episode by derivation of a second model from the original. Finally, equations for the mean displacement and mean-squared distance displacement of spores from the original point source were determined. In particular, it is shown how the latter is used for characterizing and comparing dispersal.

The developed model was tested by comparing theoretical results to experimental results obtained with a rain simulator. Data sets were used for the splash dispersal of *Colletotrichum acutatum* conidia in relation to surface topography (Yang *et al.*, 1990) and rain intensity (Madden *et al.*, 1996).

3.2 Materials and Methods

For a better understanding of the model construction, an explanation of the experimental set-up used to assess spore dispersal is given.

At the start of the experiment, spore samplers are placed at several distances around an inoculum point source. The sampler consists of a petri dish, with a semi-selective growth medium for *Colletotrichum* species, and a rain shield to prevent direct interception of rain (Fig.1 in Yang *et al.*, 1990). The point source comprises infected strawberry fruit with sporulating lesions, and the entire area receives a rain of up to 46 minutes in duration. At several times during the rain, usually every 5 min, splash droplets with spores are collected in the sampler for 1 minute. Petri dishes are then replaced and droplets are again collected. Petri dishes are incubated and fungal colonies are counted. Calibration studies show that 65% of spores deposited in the dishes germinate and form colonies (Yang *et al.*, 1990). Resulting data sets consist of number of colonies per square centimeter per minute at several times and distances during rain episodes. Detailed information about the experimental set-up is given by Madden (1992).

Data from two published experiments were considered here. In the first, dispersal (measured as colonies) was determined over three ground covers, plastic, soil, and straw, with rain intensities of 15 and 30 mm h⁻¹ (Yang *et al.*, 1990). In the second, dispersal was determined over soil for seven rain intensities, from 2 to 60 mm h⁻¹ (Madden *et al.*, 1996).

3.3 The model

3.3.1 Spatial spread of spores from a point source

Taking the above described experimental results as a starting point, the model should represent the number of spores found at an arbitrary point on the surface at any time during a rain episode.

Suppose $M(t,x,y)$ stands for the number of spores found at a particular point, having

coordinates (x,y) in relation to the source at time t during a rain episode. The number of spores entering a Petri dish at some distance from the source per unit of time is assumed to be equal in all directions around the inoculum source, because of lack of air movement in these studies. This means the number of spores will be homogeneously spread on any circle around the source. Now, the radius r of any circle around the source can be expressed as $r = \sqrt{x^2 + y^2}$. Henceforth, we use $N(t,r)$ to denote the number of spores found at any arbitrary point on a circle with radius r around the source at time t during the rain.

We first describe the spores on the surface at the start of the rain. At time $t=0$, the only spores are at the point source, $r=0$. Because, ultimately, we want to assess distances spores travel from this starting point, we need a model expression in which spores at the source do not take any space themselves. The space that is occupied by an infected fruit is small relative to the distances travelled; thus, a "zero space" expression is reasonable. A mathematical expression for such an impulse function is the Dirac delta function, denoted as $\delta(r)$ (Doucet and Sloep, 1992). This function characterizes the initial distribution of spores at $t=0$, that is, it has an area of 1 at $r=0$ and an area of 0 at all other values of r at the start of the rain event.

The entire experimental area (including the point source of spores) is then exposed to rain. It was found that spores at the source decrease exponentially in number (Madden *et al.*, 1992; Yang *et al.*, 1991^a). This can be explained from the physical mechanism with which spores are dispersed. When it starts raining, spores are incorporated in the formed splash droplets and are redistributed around the source in ballistic flight trajectories (Macdonald and McCartney, 1989). The number of spores that are splashed away is proportional to the number present at a given time, resulting in an exponential decline. This information can be translated in a second model term that represents the declining number of spores at the source once it starts raining. The Dirac delta function should then be multiplied with the number of spores at the start of the rain, denoted by $N(0,r)$, to get the actual spore numbers. If the number of spores at the source, $N(t,0)$, decreases proportional to the number present, spores have a certain probability per unit time of being splashed, denoted here by $\lambda \text{ min}^{-1}$. The exponentially decreasing spore numbers at $r=0$ can be described as

$$N(t,r) = e^{-\lambda t} \delta(r) \cdot N(0,r). \quad (3.1)$$

During its first flight from the source, a spore has a probability of landing at a place where it cannot be splashed again. This occurs when a spore lands, for example, in the straw just below the surface. This mechanism explains why fruit disease incidence is lower when straw instead of soil is used as a ground cover (Madden *et al.*, 1993).

If a spore lands at a place that is not in direct contact with impacting raindrops, it is out of the process for the rest of the rain episode. Spores have a probability of splashing out of the process each time they are impacted by a water drop. Therefore, an

expression for the probability of staying in the process per splash should be incorporated in the model, denoted by ε (unitless). The probability of being removed per splash is then $1 - \varepsilon$.

Now, consider the distance a spore can travel when it is splashed away once from the source, given that it stays in the process. Yang *et al.* (1991^a) performed an experiment in which a fruit was hit by a raindrop (of a range of diameters and impact velocities), and travel distances of the splashing water droplets were measured. A frequency distribution of droplet travel distances could be determined (Fig.7 in Yang *et al.*, 1991^a). Since spores are dispersed in these splash droplets, this experiment gives a good representation of spore travel distances from a single drop impaction.

The frequency distribution of droplet travel distances could be approximated by a normal distribution. Any other distributions could be used with tail probabilities smoothly diminishing to zero that would account for skewness of the distribution, such as a double-exponential distribution (Pielaat and Van den Bosch, 1998). However, the normal distribution is reasonably robust to some nonsymmetry of the actual distribution. As droplets are dispersed equally around the source, we need a normal distribution in two dimensions to represent droplet travel distances.

Let $D(x,y)$ be the probability density function of a spore travelling to position (x,y) from position $(0,0)$ during one splash. If, for example, x_1 and y_1 are normally distributed, both with expectation 0, and travelling in x and y direction occurs uncorrelated, then the spatial density of spores after one splash can be specified as

$$D(x_1, y_1)^{*1} = \frac{1}{2\pi\sigma^2} e^{-\frac{(x_1^2 + y_1^2)}{2\sigma^2}}, \quad (3.2)$$

in which σ^2 is the variance for the travel distance x and y (Mood *et al.*, 1950). A spore will continue splashing during the rain. Assume that each time a spore is splashed from any point on the surface, it has the same probability density function for travel distances and that during a rain episode spores splash i times. The equation for the spatial spread after being splashed i times, $D(x,y)^{*i}$, can then be derived using the above discussed method for $i=1$. Using the normal distribution for $D(x,y)$ (Mood *et al.*, 1950), it follows that

$$D(x, y)^{*i} = \frac{1}{2\pi i \sigma^2} e^{-\frac{(x^2 + y^2)}{2i\sigma^2}}. \quad (3.3)$$

Because the resulting probability distribution is a bivariate normal distribution with $\rho=0$ and so rotationally symmetrical again, the probability is a function of distance $r=\sqrt{x^2 + y^2}$ only, and we can write

$$D(r)^{*i} = \frac{1}{2\pi i \sigma^2} e^{-\frac{r^2}{2i\sigma^2}}. \quad (3.4)$$

We now have an expression for the decreasing number of spores at the source (3.1) and an expression for the spatial movement of spores in the field once they have splashed i times (3.4). The final step here is to determine the probability of being splashed i times.

Some spores are splashed more times than others in a rain episode. Therefore, a probability distribution is incorporated for the number of splashes, i per spore within time t , in which the discrete stochastic process for $i=0,1,2,\dots,\infty$. We assume that spores splash independently and, therefore, the number of splashes for a spore in time t is poisson distributed. Given that the probability of being splashed per unit time is denoted by λ , the mean number of splashes during time t equals λt . The resulting poisson probability is

$$P(i) = \frac{(\lambda t)^i e^{-\lambda t}}{i!}. \quad (3.5)$$

In Pielaat and Van den Bosch (1998), a partial differential equation was developed to describe the process of splash dispersal. Solving this model, a poisson distribution appears to represent the probability of a spore being splashed i times at some time during the rain event.

Because each time a spore is splashed it has a probability, ε , of staying in the system, we have to multiply equation 3.5 by $\varepsilon^i N(0,r)$ to determine the number of spores still in the process and splashed i times. Multiplying (3.5) times $\varepsilon^i N(0,r)$ with (3.4) gives the number of spores found at distance r from the source once they are splashed i times.

Finally, in a rain episode, different spores splash different number of times, all having a different contribution to the number of spores at a certain distance from the source. Therefore, we make a summation over all possible times a spore can be splashed ($i=1$ to ∞) to attain the actual spatial spread of spores in the field at any time during a rain event.

This leads to the resulting equation for number of spores at any arbitrary point on r at time t during the rain:

$$N(t,r) = \left[e^{-\lambda t} \delta(r) + \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i \frac{1}{2\pi i \sigma^2} e^{\frac{-r^2}{2i\sigma^2}} \right] \cdot N(0,r), \quad (3.6)$$

when the model is specified with a normal distribution for $D(r)^{**}$. Although (3.6) shows an infinite series $\sum_{i=1}^{\infty}$, the individual terms decline relatively soon to 0. For calculations, we added new terms until the contribution with respect to the previous summation differed by less than 0.001%. This was achieved with an upper summation limit of $i=99$. Dividing both sides of (3.6) by $N(0,r)$ produces the probability of a spore travelling distance r in time t , $P(t,r)$ ($P(t,r)=N[t,r]/N[0,r]$).

A mathematical derivation of this model is described in Pielaat and Van den Bosch

(1998), based on a general solution of a partial differential equation for $P(t,r)$ and an unspecified $D(r)^{*i}$.

3.3.2 Number of spores during a rain episode

To describe the total number of spores present at all distances surrounding the source, an equation can be derived from (3.6). Since now we are discussing the change in number of spores over time, only a time (but no distance) component is needed to describe what happens during a rain episode. Following the model (3.6), the initial spores at the point source decline proportional to the number present. That is, if $N_1(t)$ represents the number of spores at the source at time t , then

$$\frac{dN_1(t)}{dt} = -\lambda N_1(t), \quad (3.7)$$

with initial condition $N_1(0) = N(0,r)$.

The number of spores away from the source, $N_2(t)$, will increase proportional to the number at the source. However, in their first splash from the source, spores already have a nonzero probability of removal from the process $(1 - \varepsilon)$. Therefore, the number of spores that reach the field per unit of time is proportional to the number of spores that were splashed from the source multiplied by their probability of remaining in the process during the first splash. Once in the field, spores have a probability per splash of disappearing from the process into the ground (or, in general, out of the system). So, simultaneously with an increase, the number of spores in the field will decrease with a factor, $\lambda(1 - \varepsilon)$, proportional to the number present. The equation for $N_2(t)$ can then be written as

$$\frac{dN_2(t)}{dt} = \lambda \varepsilon N_1(t) - \lambda(1 - \varepsilon)N_2(t), \quad (3.8)$$

with initial condition $N_2(0) = 0$.

The number of spores that are removed from the field, $N_3(t)$ equals the second term in (3.8) and spores directly splashed out of the process from the source, namely,

$$\frac{dN_3(t)}{dt} = \lambda(1 - \varepsilon)N_2(t) + \lambda(1 - \varepsilon)N_1(t), \quad (3.9)$$

with initial condition $N_3(0) = 0$.

Solving the equation for $N_2(t)$ leads to

$$N_2(t) = -N(0, r)(e^{-\lambda t} - e^{-\lambda(1-\varepsilon)t}) \quad (3.10)$$

This equation shows the number of spores surrounding the source increases from the start of the rain up to $t^* = -\ln(1-\varepsilon)/\lambda\varepsilon$, and then the number decreases again.

To check the correctness of (3.10), it is derived directly from the full model (3.6) for spore dispersal. The full model (3.6) shows that during the rain, the initial number of spores at the source, $N(0, r)$, declines according to $N(0, r) e^{-\lambda t}$. Once a spore has left the initial source, it is displaced in the field as

$$N(t, r) = N(0, r) \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i D(r)^{*i}. \quad (3.11)$$

This equation shows the number of spores that travelled a particular distance at some time in a rain episode, whereas now we would like to know the total number of spores over the whole surface surrounding the source in time. To determine the total, sum the number of spores at the different distances from the source. For continuous space, this is done by integrating over all possible places a spore can be at a particular time; therefore,

$$N(t) = N(0, r) \sum_{i=1}^{\infty} \frac{(\lambda t)^i e^{-\lambda t}}{i!} \varepsilon^i \int_0^{\infty} D(r)^{*i} dr. \quad (3.12)$$

Since the sum, or integration, of the probability $D(r)^{*i}$ for all possible places is 1 by definition, the solution of (3.12) is

$$N(t) = N(0, r) e^{-\lambda t} \sum_{i=1}^{\infty} \frac{(\varepsilon \lambda t)^i}{i!} \cdot 1. \quad (3.13)$$

For following manipulation it is useful to sum from $i=0$ rather than $i=1$. Because $(\varepsilon \lambda t)^0/0! = 1$, the lower limit on the summation in (3.13) can be changed to 0 and then 1 can be subtracted to give an equivalent expression:

$$N(t) = N(0, r) e^{-\lambda t} \sum_{i=0}^{\infty} \frac{(\varepsilon \lambda t)^i}{i!} - 1. \quad (3.14)$$

The term $\sum_{i=0}^{\infty} (\varepsilon \lambda t)^i / i!$ is the Taylor series expansion of $e^{\varepsilon \lambda t}$. Substituting the exponential expression for the infinite sum results in

$$N(t) = N(0, r) e^{-\lambda t} (e^{\varepsilon \lambda t} - 1), \quad (3.15)$$

which is equal to (3.10) for $N_2(t)$.

3.3.3 Measures of distance displacement of spores during a rain

Determining distances spores travel has been a goal of experimental work using a rain simulator (Fitt *et al.*, 1992; Madden *et al.*, 1992; Reynolds *et al.*, 1987; Yang *et al.*, 1990). Resulting data sets for number of spores deposited per time unit at various distances can be used to calculate measures of displacement of spores during rains. First, the total number of spores entering a contour around the source per unit time after some period of rain can be calculated. For that, the number of spores found at each single sampling distance is multiplied by $2\pi r$, the circumference of a circle. The most obvious summary measure of distance is the mean (or expected) displacement of spores per time during the rain. That is, the mean is determined by multiplying the sampling distances by accompanying number of spores found for each contour at some time, summing up the resulting numbers, and dividing by the original number of spores at the start of the rain. But, because the initial number of spores at the source is not exactly known for these experiments, only an expected displacement of spores multiplied by the initial number ($N[0, r]$) can be calculated from the data: that is,

$$E[r(t)]N(0, r) = \sum rN(t, r)2\pi r, \quad (3.16)$$

in which, $E[r(t)]$ = the mean distance spores travel from the source, and $N(t, r)2\pi r$ = total number of spores found at a contour with radius r during some time interval in the rain.

In terms of model (3.6), the expected value for displacement of spores around the source during a rain can be specified following Doucet and Sloep (1992) as

$$E[r(t)]N(0, r) = \int_0^\infty rN(t, r)2\pi r dr. \quad (3.17)$$

That is, instead of summing up discrete distances (3.16), we integrate over all possible locations of the continuous distance variable r . Using (3.6) for $N(t, r)$ in (3.17), we find

$$E[r(t)]N(0, r) = e^{-\lambda t} \frac{1}{2\sqrt{2\pi\sigma^2}} \sum_{i=1}^{\infty} \frac{(\varepsilon\lambda t)^i}{i!} \sqrt{i} N(0, r). \quad (3.18)$$

Equation (3.18), with parameter values estimated (described in Results) or assumed, can be related to experimental data on spore deposition using calculated $E[r(t)]$ from (3.16). A different expression would result for (3.18) if an other $D(i)^{**}$ function was used in (3.6).

For many continuous processes in space, it is easier to derive the mean-squared displacement, $E(r^2)$ (Othmer *et al.*, 1988):

$$E[r^2(t)]N(0, r) = \sum r^2 N(t, r) 2\pi r. \quad (3.19)$$

The equation for the model of $N(t, r)$ (3.6) then changes to

$$E[r^2(t)]N(0, r) = \int_0^\infty r^2 N(t, r) 2\pi r dr, \quad (3.20)$$

which leads to

$$E[r^2(t)]N(0, r) = 2\varepsilon\lambda\sigma^2 t e^{-\lambda(1-\varepsilon)t} N(0, r). \quad (3.21)$$

Equation (3.21) specifies the mean-squared displacement of spores in a rain episode times the original number of spores at the source. Spores will reach their maximum squared distance at $t^* = 1/\lambda(1 - \varepsilon)$. A striking result is that the mean-squared displacement of spores, $2\varepsilon\lambda\sigma^2 t e^{-\lambda(1-\varepsilon)t}$, is independent of the complicated spore travel distribution D^{**} and requires no infinite summation. In fact, (3.21) is valid for all D^{**} functions. Therefore, it will in many cases be easier to derive and use this quantity than the mean displacement (3.18), which does depend on D^{**} . Again, (3.21) can be related to experimental data using calculated $E[r^2(t)]$ from (3.19).

3.4 Parameter estimation

The full model for splash dispersal, (3.6), in principle could be used to estimate parameters. However, the infinite summation, even approximated by a series from 1 to 99 (or less), is difficult to use in fitting the equation to $N(t, r)$ data. Initial efforts to directly use (3.6) on the ground cover (Yang *et al.*, 1990) and rain intensity (Madden *et al.*, 1996) data sets resulted in highly correlated and unreliable parameter estimates. Thus, equations for $N(t)$ and $E[r^2(t)]$, (3.15) and (3.21), respectively, were used simultaneously to estimate parameters. To use (3.15) and (3.21), the observed $N(t, r)$ data were adjusted to obtain $N(t)$ and $E[r^2(t)]$, as described below.

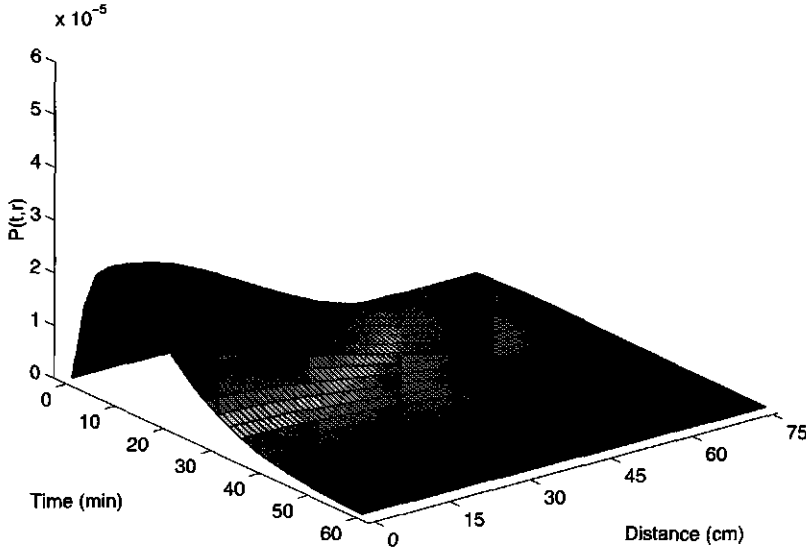


Figure 3.1: Probability of a spore travelling distance r (cm) from a point source of inoculum over time t (min) during a rain event, $P(t,r)$, with $P(t,r) = N(t,r)/N(0,r)$ and $N(0,r)$ being the number of spores at the source (based on 3.6). The probability of being splashed per unit of time, λ , is 0.13 min^{-1} ; the probability of staying in the process per splash event, ε , is 0.25; and the standard deviation for $D(r)^{*i}$ (the flight distribution of spores for individual splash events), σ , is 25 cm.

The full model or the pair of models contains four parameters, $N(0,r)$, λ , ε , and σ . Number of spores at the point source ($N[0,r]$) was controlled in the experiments described here and was considered to be fixed for all treatments (ground covers or intensities) in an experiment (Madden *et al.*, 1996; Yang *et al.*, 1990). Here, we use $N(0,r) = 10^5$ for rain intensity study and $N(0,r) = 10^6$ for the ground cover experiment. The other three parameters were estimated for each treatment using nonlinear least squares regression.

Data adaptations. To calculate the total number of spores in the process at some time during a rain episode, $N(t)$, using (3.15), the total number of colonies from 0 to the largest radius should be counted. However, only the number of spores per square centimeter per minute at particular distances were determined (Madden *et al.*, 1996; Yang *et al.*, 1990). We assumed that the counted number of spores per square centimeter at some distance is constant at all locations within an annulus around the source. For example, if spore numbers $N(t,r)$ were measured at 20, 40, 60 and 80 cm from the

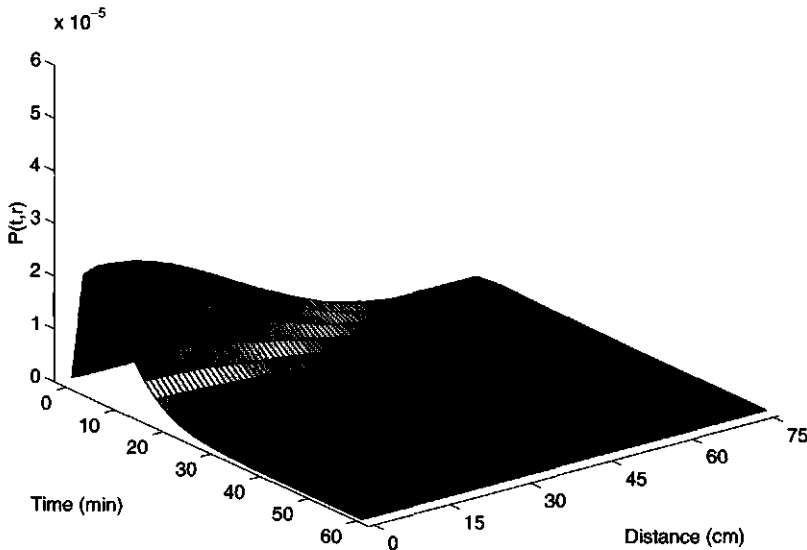


Figure 3.2: Probability of a spore travelling distance r (cm) from a point source of inoculum over time t (min) during a rain event, $P(t,r)$, with $P(t,r) = N(t,r)/N(0,r)$ and $N(0,r)$ being the number of spores at the source (based on 3.6). The probability of being splashed per unit of time, λ , is 0.25 min^{-1} ; the probability of staying in the process per splash event, ϵ , is 0.25; and the standard deviation for $D(r)^{**}$ (the flight distribution of spores for individual splash events), σ , is 25 cm.

source, then the total numbers within a radius (r) of 20 cm from the source is estimated as $N(t,20) \cdot \pi \cdot (20 + 10)^2$. Subsequently, the total number between 20 and 40 cm from the source is estimated as $N(t,40) \cdot \pi \cdot (40 + 10)^2 - N(t,20) \cdot \pi \cdot (40 - 10)^2$, and so on. Adding up the results gives the total number of colonies within a radius of 80 cm from the source at some time during the rain.

For each consecutive time in the rain, the mean displacement or mean squared displacement of spores multiplied by the initial number can, in principle, be calculated from the data according to (3.16) or (3.19). However, as discussed in the above paragraph, only data at specified locations were obtained. Thus, it was assumed that $N(t,r)$ was constant from $r - \Delta r$ to $r + \Delta r$ (with $\Delta r = 10$ cm here). That is, for $r = 20$, the formula within the summation of (3.16) becomes $rN(t,30)2\pi r$; and for $r = 40$, $r(N(t,50) - N(t,30))2\pi r$.

Statistical analysis. Equations (3.15) and (3.21) were simultaneously fitted to the observed data for $N(t)$ and $E[r^2(t)]$, respectively, using nonlinear least-squares regression

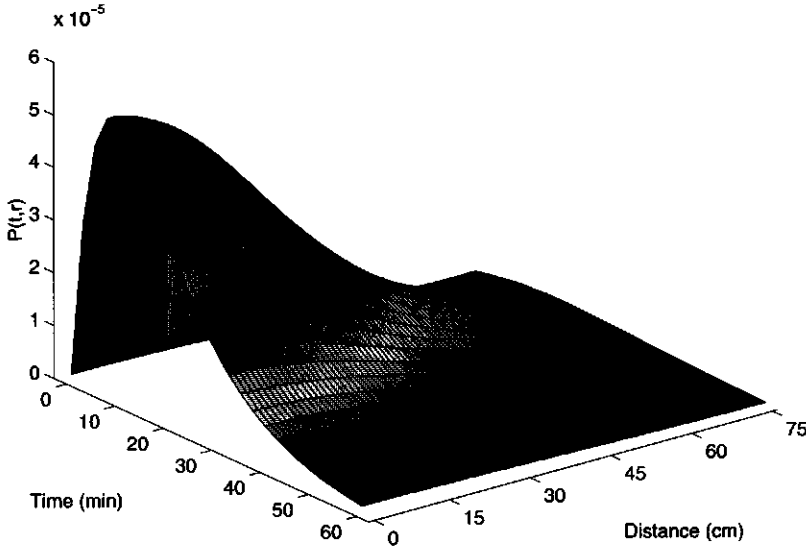


Figure 3.3: Probability of a spore travelling distance r (cm) from a point source of inoculum over time t (min) during a rain event, $P(t,r)$, with $P(t,r) = N(t,r)/N(0,r)$ and $N(0,r)$ being the number of spores at the source (based on 3.6). The probability of being splashed per unit of time, λ , is 0.13 min^{-1} ; the probability of staying in the process per splash event, ε , is 0.50; and the standard deviation for $D(r)^{*i}$ (the flight distribution of spores for individual splash events), σ , is 25 cm.

(Seber and Wild 1989). The Gauss-Newton method for minimization of the generalized sum or squares of the residuals (across the two equations) was used in conjunction with the "seemingly unrelated regression (SUR)" parameter estimation method. Calculations were performed with the MODEL procedure of SAS/ETS (Statistical Analysis System 6.12; SAS Institute Cary, NC). MODEL determines the analytical partial derivatives of the models with respect to the parameters that are needed in the Gauss-Newton procedure. This procedure can be used for systems of nonlinear equations in which one or more of the parameters are shared by two or more equations. It was assumed that the errors (unexplained variation) for each equation (estimated by the residuals) were identically and independently distributed with zero means and a positive definite covariance matrix. The SUR method allows for the nonzero correlation of the residuals across the equations in determining parameters and their estimated (asymptotic) standard errors.

For the rain intensity study (Madden *et al.*, 1996), certain functions of the estimated

Table 3.1: Estimated parameters of (3.15) and (3.21) for splash dispersal of spores with seven rain intensities. Data are from Madden *et al.* (1996). For equations, it was assumed that $N(0,r)=10^5$.

Rain Intensity (mm h ⁻¹)	Estimated parameters			Root MSE ^a	
	λ (min ⁻¹)	ε (splash ⁻¹)	σ (cm)	N(t)	r ² (t)
2	0.023 (0.010) ^b	0.0002 (0.0004)	23 (5.8)	2.5	1.3
4 ^c	0.002 (0.031)	0.033 (4.1)	20 (5.2)	21	21
7	0.034 (0.010)	0.046 (0.005)	20 (1.0)	54	49
11	0.026 (0.007)	0.130 (0.012)	15 (0.2)	951	48
15	0.075 (0.013)	0.210 (0.025)	26 (2.2)	2448	756
30	0.130 (0.015)	0.460 (0.031)	20 (1.3)	3600	982
60	0.116 (0.014)	0.480 (0.033)	18 (1.4)	4166	521

^aRoot mean square error of the observed values around the predicted curves.

^bStandard error of estimated parameter in parentheses.

^cModel-fitting procedure did not converge.

parameters were related to intensity (I ; mm h⁻¹) using a nonlinear empirical model. The model used was either $\ln(y) = a - be^{-cI}$ or $y = a - be^{-cI}$, in which a , b , and c are parameters, and y is some combination of the model parameters (e.g., $\lambda[1 - \varepsilon]$).

3.5 Results

3.5.1 Model simulation

To give a general insight into the model, some simulation results will be shown first. After the start of a rain spores gradually move into the area surrounding the point source. Figure 3.1 shows a model simulation for $P(t,r)$ ($= N[t,r] / N[0,r]$) with a set of reasonable parameter values (as shown below). Here, $P(t,r)$ increases at least up to approximately 10 min at all distances from the source. Beyond this time, $P(t,r)$ decreases. This decreasing probability is primarily due to depletion of spores at the

Table 3.2: Estimated parameters of (3.15) and (3.21) for splash dispersal of spores with three ground covers at two rain intensities. Data are from Yang *et al.* (1990). For equations, it was assumed that $N(0,r)=10^6$.

Ground Cover	Rain Intensity (mm h ⁻¹)	Estimated parameters			Root MSE ^a	
		λ (min ⁻¹)	ε (splash ⁻¹)	σ (cm)	N(t)	r ² (t)
Plastic	15	0.12 (0.008) ^b	0.05 (0.003)	32 (2.2)	1826	1159
Soil		0.09 (0.02)	0.05 (0.007)	36 (1.6)	5335	215
Straw		0.12 (0.022)	0.03 (0.006)	32 (2.2)	3873	969
Plastic	30	0.20 (0.012)	0.19 (0.012)	27 (0.7)	6610	1725
Soil		0.13 (0.005)	0.10 (0.005)	29 (3.0)	2528	2636
Straw		0.25 (0.018)	0.09 (0.007)	20 (1.0)	3060	585

^aRoot mean square error of the observed values around the predicted curves.

^bStandard error of estimated parameter in parentheses.

inoculum source and removal of spores from the system. Figure 3.2 shows a model simulation in which λ is set to a larger value. When λ increases, it takes spores less time to be spread to the area surrounding the source, and the probability of being splashed to larger distances in relatively little time increases compared with Figure 3.1. Setting ε to a larger value increases $P(t,r)$ in time for all distances (Figure 3.3).

3.5.2 Parameter estimates

Table 3.1 shows the estimates and accompanying (asymptotic) standard errors for λ , ε and σ when all three parameters were simultaneously estimated for the rain intensity data of Madden *et al.*, (1996).

The nonlinear least squares procedure converged for all rain intensities except 4 mm h⁻¹, although parameter estimates were reasonable for this intensity as well. Spore deposition was very low (but nonzero) at the lowest rain intensities (Fig.2 in Madden *et al.*, 1996), and there probably was insufficient change in $N(t,r)$ with increasing t and r (or change in $N(t)$ with increasing t) for the least squares procedure to converge at 4 mm h⁻¹. Standard errors were very small except for the lack of convergence case.

Correlations of the estimated parameters were all under 0.75, and most were less than 0.5.

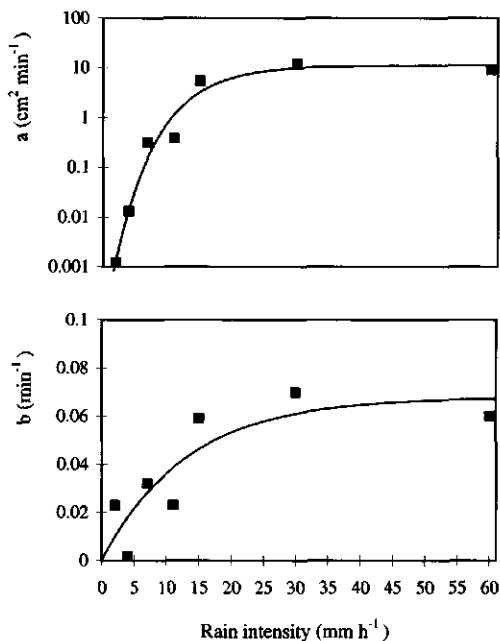


Figure 3.4: Estimated dispersal coefficient ($\alpha = \varepsilon \lambda \sigma^2 / 2$) and the rate of spore loss ($\beta = \lambda [1 - \varepsilon]$) for increasing rain intensities (I ; mm h⁻¹). Values for α , based on estimated parameters in Table 3.1, could be fitted by a limited exponential growth model the form $\ln(\alpha) = a - b e^{-cI}$, in which $a = 2.39$ (standard error [SE] = 0.51), $b = 12.30$ (SE = 1.24) and $c = 0.15$ (SE = 0.03). For β , the model $\beta = a - b e^{-cI}$ was used based on estimated parameters in Table 3.1, in which $a = 0.068$ (SE = 0.016), $b = 0.068$ (SE = 0.020), and $c = 0.076$ (SE = 0.062).

There were clear trends in parameter estimates with increasing intensity. Except for 11 mm h⁻¹, the probability of being splashed per unit time, λ , increased with rain intensity up to 30 mm h⁻¹. Because the number and size of both impacting drops and splashing droplets increases with increasing rain intensity (Madden *et al.*, 1996), λ was expected to follow this trend. For 60 mm h⁻¹, estimated λ was slightly less than for 30 mm h⁻¹, but the difference was not significant, suggesting that a maximum λ was reached. The increase in water puddles at the highest intensity (Madden, unpublished data) may dilute the surface density of spores per area, thus decreasing the probability of splash. The estimated probability of staying in the process per splash, ε , also increased with rain intensity. At intensities of ≤ 7 mm h⁻¹, ε was less than 0.05, meaning that the probability of being removed per splash ($1 - \varepsilon$) was greater than 0.95. The estimated

measure of distance a spore splashes, σ , was invariant to intensity (Table 3.1). That is, $\sigma \approx 20$ cm at all intensities.

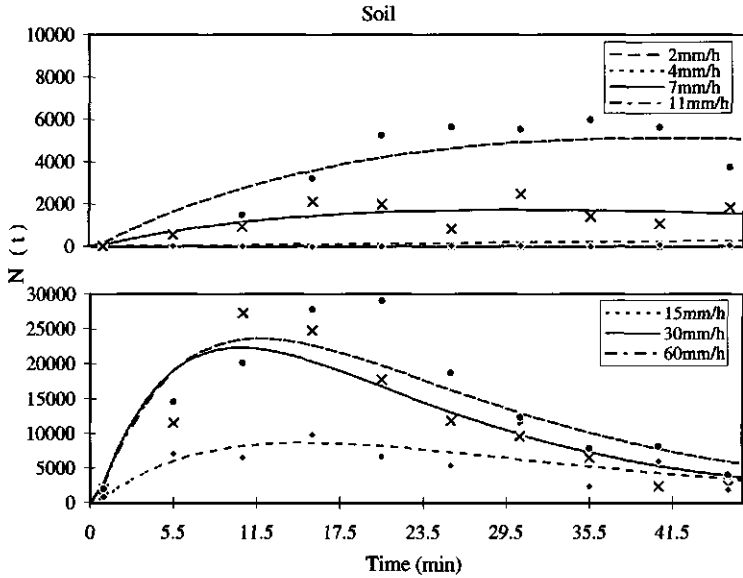


Figure 3.5: Change in total number of spores across a circular area (with a radius of 100 cm), $N(t)$, during a rain event for seven different intensities using soil as a ground cover. Symbols used for observed values are triangles for 2 mm h⁻¹, diamonds for 4 mm h⁻¹ and 15 mm h⁻¹, crosses for 7 and 30 mm h⁻¹, dots for 11 and 60 mm h⁻¹. Data are from Madden *et al.* (1996) for number of spores per square centimeter per minute at various times (t) and distances (r) from the source, $N(t,r)$. Curves are based on eq. 3.15 with parameter estimates in Table 3.1, with an assumed source strength ($N[0,r]$) of 10^5 spores.

In Figure 3.4 the so-called “diffusion” coefficient ($\alpha = \lambda \varepsilon \sigma^2 / 2$), and β is the rate of spore loss ($\beta = \lambda [1 - \varepsilon]$) (Pielaat and Van den Bosch, 1998) are plotted versus rain intensity. There was a general increase in α and β with intensity at low intensities, with a leveling off at ≥ 30 mm h⁻¹.

Table 3.2 shows the estimated parameter values and accompanying (asymptotic) standard errors for the ground cover data of Yang *et al.* (1990), when three parameters were simultaneously estimated.

As with the intensity experiment (Madden *et al.*, 1996) described above, both λ and ε increased when rain intensity increased from 15 to 30 mm h⁻¹. Estimated σ decreased slightly with increasing intensity; however, the values of σ at 30 mm h⁻¹ were similar to those determined in the intensity experiment (Table 3.1). Furthermore, λ for soil at 15

and 30 mm h^{-1} with the ground cover experiment (Table 3.2) were very similar to the estimates for these intensities with the rain intensity experiment. Surprisingly, λ was larger for straw than for soil. For the 30 mm h^{-1} rain intensity, the probability of staying in the process per splash, ε , was largest when plastic was used as ground cover ($\varepsilon \approx 0.2$) and smallest when straw was used ($\varepsilon \approx 0.1$). For 15 mm h^{-1} this trend was somewhat less clear and ε was only affected by straw (0.05 versus 0.03). However, the lower values overall for ε at this intensity make it more difficult to quantify differences. At an intensity of 30 mm h^{-1} , the estimated σ for plastic and soil ($\sigma \approx 28 \text{ cm}$) were greater than for straw ($\sigma \approx 20$). At 15 mm h^{-1} , σ was not greatly affected by ground cover. From this study it can be seen that the effect of ground cover on dispersal components is most discernible at high rain intensity.

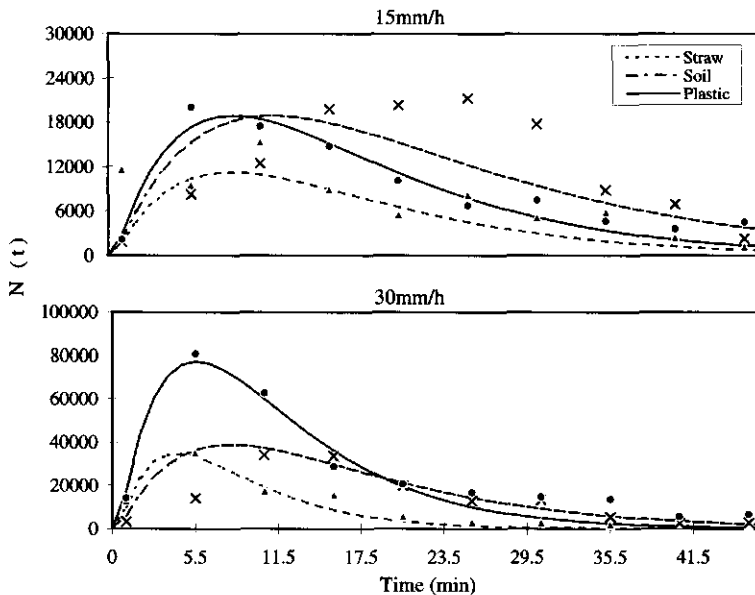


Figure 3.6: Change in total number of spores across a circular area (with a radius of 120 cm), $N(t)$, during a rain event for two different labeled intensities using straw, soil or plastic as a ground cover. Symbols used for observed values are triangles for straw, crosses for soil and dots for plastic. Data are from Yang *et al.* (1990) for number of spores per square centimeter per minute at various times (t) and distances (r) from the source, $N(t,r)$. Curves are based on (3.15) with parameter estimates in Table 3.2, with an assumed source strength ($N[0,r]$) of 10^6 spores.

3.5.3 Number of spores

According to (3.15) and reasonable parameter values, total number of spores in the field at time t , $N(t)$, should initially increase during a rain episode.

This trend can be seen in Figures 3.5 and 3.6 when the model is applied to experimental data sets. Figure 3.5 shows $N(t)$ over time for the data sets with different rain intensities (Madden *et al.*, 1996), all with the same ground cover. Because λ generally increased with intensity (Table 3.1), the maximum number of spores was predicted to be reached after a relatively short period of rain at high rain intensity compared to low intensity (at $t^* = -\ln(1-\varepsilon)/\lambda\varepsilon$). Also, $N(t)$ overall was predicted to increase with intensities because of the term in (3.15), at least until spore removal (reflected by ε) dominates over the number of spores leaving the source. Both data and model outcome agree with these general expectations (Figure 3.5). Only from 30 to 60 mm h⁻¹ did $N(t)$ fail to consistently increase with intensity, probably because λ did not increase. For the low rain intensities (< 15 mm h⁻¹), the probability of being splashed was small (Table 3.1), and $N(t)$ was still increasing even after 45 minutes of rain (Figure 3.5). For the high rain intensities, the maximum number of spores splashed across an area was reached quickly, and ε then dominates the process.

If plastic is used as a ground cover (Figure 3.6), more spores are expected to stay in the process compared to other ground covers, causing a larger $N(t)$ than for soil or straw (Madden, 1992; Yang *et al.*, 1990). This was clearly seen for 30 mm h⁻¹ and, to a lesser extent, for 15 mm h⁻¹ (where there was only a slight difference between plastic or soil versus straw). These results can be predicted from the model because of the large ε for plastic compared to the other covers at 30 mm h⁻¹ (Table 3.2). At the lower intensity, ε for plastic was slightly higher than for straw, but not for soil, although all values were low for ε . At 30 mm h⁻¹, $N(t)$ for soil and straw were similar at low t , and then soil had the larger $N(t)$, presumably because of the combined effects of ε and λ . At 15 mm h⁻¹, straw clearly had the lowest $N(t)$ of the three ground covers, which can be explained by the small, but significant, difference in ε between straw and plastic.

3.5.4 Measures of displacement

Figures 3.7 and 3.8 illustrates data and model predictions for the mean squared travel distance times $N(0,r)$ for different intensities and ground covers (Yang *et al.*, 1990; Madden *et al.*, 1996). In general, $E(r^2)$ increased with most rain intensities, and the time of the maximum $E(r^2)$ was shorter for high intensity rains than low intensity ones. Also, surface topography affected the magnitude of $E(r^2)$, especially at 30 mm h⁻¹. Model results using parameter estimates of Tables 3.1 and 3.2 agree with the observations. The figures show that during a rain episode, observed mean squared displacement first increased and then decreased. Spores being lost from the system, that is, $\varepsilon < 1$, causes this result in the model. In fact, if $\varepsilon = 1$, $E(r^2)$ reduces to the linear function $2\lambda\sigma^2t$.

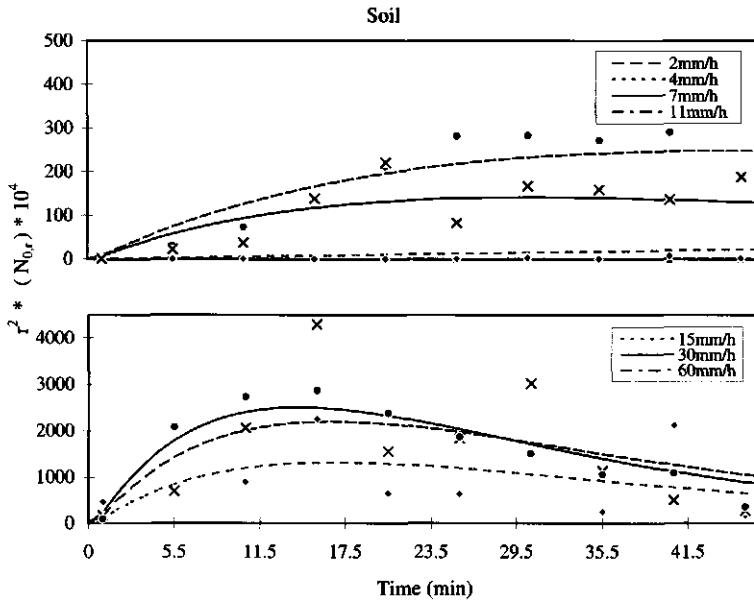


Figure 3.7: Mean squared distance spores travel times initial number, $E(r^2)N(0,r)$, during a rain event for seven different intensities using soil as a ground cover. Symbols used for observed values are triangles for 2 mm h^{-1} , diamonds for 4 and 15 mm h^{-1} , crosses for 7 and 30 mm h^{-1} , dots for 11 and 60 mm h^{-1} . Data are from Madden *et al.* (1996) for number of spores per square centimeter per minute at various times (t) and distances (r) from the source, $N(t,r)$. Observed $E(r^2)N(0,r)$ was calculated with (3.19). Curves are based on (3.21) with parameter estimates in Table 3.1, with an assumed source strength ($N(0,r)$) of 10^5 spores.

Because λ generally increased with intensity (Table 3.1), the distance spores traveled by a certain time was also expected to increase and then decrease again as spores are removed. This can be seen in Figure 3.7. Maximum values for $E(r^2)$ ranged from about 400 cm^2 ($[E(r^2)]^{1/2} \approx 20 \text{ cm}$) for a 30 mm h^{-1} rain down to $< 10 \text{ cm}^2$ ($[E(r^2)]^{1/2} < 3 \text{ cm}$) for intensities under 5 mm h^{-1} .

Ground cover had a major effect on $E(r^2)$ (Figure 3.8). Maximum values for $E(r^2)$ ranged from about 30 cm^2 ($[E(r^2)]^{1/2} \approx 5 \text{ cm}$) for straw to about 125 cm^2 ($[E(r^2)]^{1/2} \approx 11 \text{ cm}$) for plastic at 30 mm h^{-1} . At the lower rain intensity, $E(r^2)$ was more similar among the ground covers, with some observed values greater for soil than for plastic. In agreement with this observation, estimated parameters were more similar among the ground covers.

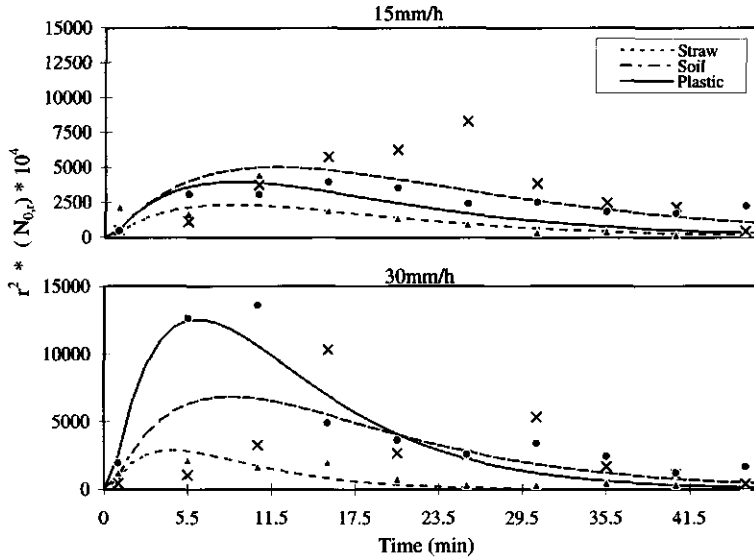


Figure 3.8: Mean squared distance spores travel times initial number, $E(r^2)N(0,r)$, during a rain event for two different intensities using straw, soil, plastic as a ground cover. Symbols used for observed values are triangles for straw, crosses for soil and dots for plastic. Data are from Yang *et al.* (1990) for the number of spores per square centimeter per minute at various times (t) and distances (r) from the source, $N(t,r)$. Observed $E(r^2)N(0,r)$ was calculated with (3.19). Curves are based on (3.21) with parameter estimates in Table 3.2, with an assumed source strength ($N(0,r)$) of 10^6 spores.

3.6 Discussion

A model was proposed here (3.6) to represent the spatial spread of spores by rain-splash from a point source at any time during a rain event over a homogeneous surface. The model was based on *i.* A probability per unit time of a spore being splashed, *ii.* A probability per splash event of a spore being removed from the dispersal process, and *iii.* A probability function for the distance a spore travels during a single splash event. A nonlinear least squares analysis for systems of equations was successfully used to fit the model to the experimental data. Results showed the model was applicable for characterizing splash dispersal under a range of conditions including situations with small and large numbers of dispersed spores.

Although the full model developed here for $N(t,r)$ (3.6) reproduces the general pattern

of spore deposition in relation to time and distance (Fig. 3.1 compared with Fig. 3.2 in literature citation Madden *et al.* 1986), the model is fairly complicated, including an infinite summation. However, composite variables were derived that are of direct biological relevance and are represented by relatively simple equations. For instance, integration over distance resulted in a description of the change in total number of spores in the area surrounding the source in time ($N(t)$; 3.15). This equation shows that $N(t)$ increases to a maximum (dependent on λ and ε) and then declines. This is because, in the model, the supply of new spores into the field from the source will predominate over the numbers being splashed out of the process (removed) during the early period of rain. Eventually, depletion of spores at the source will occur (3.1) and, ultimately, few spores will be added to the total number already present in the field. At this later time, the number of spores being splashed out of the process will predominate over new spores from the source, and the predicted $N(t)$ will decrease. Equation (3.15) also gives insight in the time at which $N(t)$ reaches a maximum for different conditions. Testing (3.15) with experimental data showed that the model followed the same general trend as the experimental results, although the predicted $N(t)$ did not reach the same maxima as the observed $N(t)$ (Figures 3.5 and 3.6) in some cases. As expected, the maximum number of spores over the area increased with rain intensity (Figure 3.5); also, the maximum number was greatly affected by surface topography, with the largest values for plastic (Figure 3.6) and lowest values for the rougher straw.

In addition to a function for $N(t)$, models were derived from (3.6) for the mean ($E(r)$; 3.18) and mean-squared ($E(r^2)$; 3.21) distances that the population of spores travels in splash droplets from the inoculum source during rain episodes, multiplied by $N(0,r)$. $E(r^2)$ is used frequently to describe spatial spread in biological systems (Othmer *et al.*, 1988), partly because the mathematical function for this variable is often a relatively simple expression. In fact, $E(r)$ cannot always be derived from spread or dispersal models. $E(r^2)$ emphasizes the population of spores that have traveled a distance somewhat above the mean, that is, $E(r^2) = \text{variance}(r) + [E(r)]^2$. Thus, $[E(r^2)]^{1/2}$ is always greater than $E(r)$. Because those spores that travel the larger distances are responsible for disease spread into new areas, $E(r^2)$ (or $[E(r^2)]^{1/2}$) gives a good impression of the potential disease spread in a crop. Although the expression for $E(r)$ was very complicated, including the D^{*i} term, the function for $E(r^2)$ was simple (3.21) and easily interpreted. Specifically, predicted $E(r^2)$ was directly related to the product of λ , ε , and σ^2 , all of which have direct physical meaning. Early in a rain event, the $E(r^2)$ predicted from the model increases over time with a slope determined by $2\lambda\varepsilon\sigma^2$. However, because of the multiplication of this product with the exponential term (3.21), $E(r^2)$ reaches a maximum at a time that depends on λ and ε (when $\varepsilon < 1$). Model results in Figures 3.7 and 3.8 can be explained by considering the splash of spores near and far from the source, early and late during a period of rain. Early in a rain event, most spores would be close to the source because splashes are of short distance (Yang *et al.*, 1991) and

droplets move in all directions. The rare spores at large distances would have arrived by one or a few long-distance splashes. Because at this early stage a spore would be splashed relatively few times, removal from the system does not dominate and mean-squared displacement is small. Over time, as the number of splashes increases, there is a net increase in $E(r^2)$ for a while as some spores move to greater distances, mostly in many separate and short-distance splashes. However, some spores are removed with every splash (when $\varepsilon < 1$), and the probability of a spore remaining in the system after many splashes is small. Spores that could have reached large distances with sufficient time if $\varepsilon = 1$ would be removed, and most spores in the area would be those close to the source that had splashed just a few times. This would correspond to a decrease in $E(r^2)$. The exact time when $E(r^2)$ decreases would depend on all the components of the dispersal process, as summarized by the parameters λ , ε , and σ (3.21). Model results were in general agreement with observed data for the rain intensity and ground cover experiments (Figures 3.7 and 3.8), with the magnitude of $E(r^2)$ and time of maximum $E(r^2)$ being dependent on experimental conditions.

In a previous paper, Yang *et al.* (1991) developed a generalized diffusion model for describing spore dispersal by rain-splash. This diffusion equation was subsequently used by Madden *et al.* (1996) and Ntahimpera *et al.* (1997) as the standard model for characterizing dispersal with the rain simulator. Pielaat and Van den Bosch (1998) recently showed that the diffusion model is a special case of equation (3.6), with combined parameters of our model being analogous to the parameter values α and β previously used in the diffusion equation for splash dispersal (Figure 3.4). However, the diffusion approximation appears only to be valid in the limit as $\lambda \rightarrow \infty$, $\varepsilon \rightarrow 1$ and $\sigma \rightarrow 0$ (Pielaat and Van den Bosch, 1998). A disadvantage of the diffusion model is that $E(r^2)$ is a monotonically increasing function of time, which is not consistent with the observed results (Figures 3.7 and 3.8) and predictions from equation (3.6). Thus, the model proposed here is an improvement over the commonly used diffusion model, both in terms of generality and describing observed data.

Factors influencing the splash-dispersal process could be captured by changing one or more of the parameter values of equation (3.6). On an individual basis, a consistent trend was found for several estimated parameters in relation to rain intensity or surface roughness. Both the probability of being splashed, λ , and the probability of staying in the process, ε , increased with rain intensity (Table 3.1). The low rate of splashing (λ) for low-intensity rains is consistent with the premise that few spores were removed per unit time at these intensities (because few were splashed), but those that were splashed were mostly removed from the process. The trend between ε and intensity may reflect the formation and depth of a water layer on the soil. That is, at low intensity, there is relatively little water on the surface (L.V. Madden, unpublished data), and we postulate that most splashed spores may be lost through the soil pores. At high rain intensity, saturation of soil with water is approached and eventually reached. This

means puddles are being formed. Under these conditions, we believe spores would be more likely to splash from puddle to puddle and not be removed from the system through pores. Therefore, ε would increase with rain intensity.

Increasing surface roughness (e.g., plastic versus straw) was characterized by decreasing estimates of ε and σ , primarily at high rain intensities (Table 3.2). This result agrees with the premise that more spores will stay in the process when a less permeable ground cover is used (Yang *et al.*, 1990). Because plastic has a relatively impermeable structure, spores can travel over larger distances (in individual splashes or in total over many splashes) with this surface compared with straw (Yang and Madden, 1993). This could be due to larger σ or ε for plastic (Table 3.2), producing larger mean-squared displacement of spores during a rain event for plastic than for straw. As σ is directly related in the model development to distances spores splash in individual splash events, it is likely, based on parameter estimates, that spores move over a larger distance when splashed from plastic and soil than when splashed from straw. In this scenario, soil would have an intermediate $E(r^2)$, which was found for 30 mm h⁻¹ (Figure 3.8). The large difference in ε between plastic and soil (Table 3.2) at this high intensity could explain the observed result.

Although we placed no a priori conditions on the parameter estimates, other than a zero lower boundary (and an upper boundary of 1 for ε), estimated values were consistent with results from independent studies on spores or water movement. For instance, Madden *et al.* (1996), in independent studies from those used here, determined the rate of spore removal directly from the source fruit (rather than based on spore deposition away from the source), essentially using equation (3.1). Rate of removal in this setting is equivalent to the probability per unit time of being splashed. They found that the estimated rate of removal for a 60 mm h⁻¹ rain was about 0.12 min⁻¹, essentially the same as our result of $\lambda=0.116$ min⁻¹ at the same intensity. They also found an increase in removal rate with increasing intensity. Furthermore, using individual drop impaction data (Yang *et al.*, 1991) and known properties of drop size distributions for natural rains, Madden *et al.* (1996) predicted that the mean flight distance of the population splash droplets from strawberry fruit (roughly related to σ) would increase only slowly with rain intensity above 10 mm h⁻¹ (Fig. 4E in Madden *et al.*, 1996). Thus, with inherent experimental variation, it is not surprising that estimated σ was stable with rain intensity in this study (Table 3.1). In a separate study, Yang and Madden (1993) directly assessed the transport of splashed water droplets across three ground covers, without spore dispersal and with minimal resplashing. Assuming an exponential distribution of droplet flights (Yang and Madden, 1993), calculated σ values of Yang and Madden (Table 4 from Yang and Madden, 1993) were of the same magnitude as our estimated σ values. Moreover, water droplet-based σ values decreased as surface roughness increased from plastic to straw (Yang and Madden, 1993), as found here for spore movement at 30 mm h⁻¹ (Table 3.2).

Although the full model for $N(t,r)$ (3.6) was of direct use in describing splash dispersal from a point source under controlled and fairly uniform conditions, this model and those derived from it ($N(t)$; equation 3.15; and $E(r^2)$; equation 3.21) may be equally useful in risk assessment under natural conditions. That is, because approximate values can be assigned to $N(0,r)$, λ , ε and σ based on controlled studies, the magnitude of $N(t)$ and $E(r^2)$ can then be predicted under various field situations. For instance, the risk of disease spread for different recorded rain intensities based on assumed parameter values. Of course, calculated $N(t)$ and $E(r^2)$ would serve more as indices of the magnitude of spore number and distance displacement than as actual predictions with this approach. In subsequent research, we will expand the full model to represent spore movement within and across a plant canopy to more fully describe field conditions under less homogeneous conditions. Other research will focus on the spore transport distribution, D^{*i} (3.4). Because rainfall consists of a distribution of drop sizes (Madden, 1992), and each drop impact potentially can produce a distribution of splash droplets containing spores, with the numbers dependent on the properties of the raindrops and surface (Yang *et al.*, 1991) work is needed to more fully characterize the link between rain and spore movement. Incorporating rainfall distribution attributes into D^{*i} should give further insight into splash dispersal and possibly improve the model predictions of $N(t)$ and $E(r^2)$.

Acknowledgements

The investigations for project 05-39-181 were supported by the Life Sciences Foundation (SLW), which is subsidized by the Netherlands Organization for scientific research (NWO). Additionally, the work of L.V. Madden is supported by the U.S. Department of Agriculture grant 95-37303-1788. We thank M.J. Jeger for helpful discussions on the subject treated in this paper and F. van den Bosch for valuable discussions and critically reading the manuscript. A. Pielaat also thanks the whole plant epidemiology group of the Ohio State University in Wooster, especially L. Wilson, W. Turechek, M. Lipps, J. Shorts, N. Ntahimpera, and B. Ellis for their hospitality and N. Ntahimpera, and B. Ellis for showing her how to get soaked from a rain simulator.

Chapter 4

Trajectories of splash droplets during splash of *Pyrenopeziza brassicae* (light leaf spot) conidia from oilseed rape plant parts

A. Pielaat D.C.A. Marshall H.A. McCartney F. van den Bosch B.D.L. Fitt¹

Abstract

The spatial spread, by rain-splash, of conidia of *Pyrenopeziza brassicae*, the cause of light leaf spot in oilseed rape, was studied in the rain tower at the Institute of Arable Crop Research, Harpenden, UK. The percentage of conidia dispersed by primary and secondary splash was assessed in separate experiments. Ballistic trajectories were determined for droplets splashing from the ground, the base or tip of a leaf, a flower or a pod in experiments using single drops of different diameters. Most of the conidia available were dispersed during simulated rain of 15 min duration. The mean horizontal distance travelled by splash droplets decreased with increasing diameter of the incident drop impacting on the different plant parts. The trajectory of a droplet depended on the plant part it splashed from. Maximum splash height ranged from 0.3 cm for droplets dispersed

¹Plant Pathology (submitted)

from a flower up to 57 cm for droplets splashed from a pod.

4.1 Introduction

The infective propagules of many plant pathogens are dispersed by rain-splash, which is considered to be the second most important natural agent, after wind, in the dispersal of spores of plant pathogenic fungi (Fitt and McCartney, 1986^a). The initial splash, which removes spores from the infectious site is termed primary splash. Once deposited, spores may be further dispersed by other splash events; these are termed secondary splash. Through many cycles of secondary splash, epidemics may be spread over distances much greater than those travelled by droplets in a single splash event. Many organisms (i.e. fungi, bacteria and nematodes) have evolved to exploit this method of spatial dispersal.

Light leaf spot of winter oilseed rape (*Brassica napus*), caused by the fungus *Pyrenopeziza brassicae* is responsible for annual losses ranging from £13M to £45M in crops in the UK (Fitt et al., 1998). The fungus can reproduce both sexually and asexually. The sexual phase produces ascospores dispersed by wind, while the asexual phase produces conidia dispersed by rain-splash (McCartney and Lacey, 1990). In winter oilseed rape crops in the UK the asexual phase of the disease appears to be responsible for most of the disease spread within the crop, especially during the winter and spring. Knowledge of the dispersal of the pathogen will improve understanding of the development of epidemics and the efficiency of the control of this disease.

This paper reports the results of a study on the spatial spread of *P. brassicae* conidia from oilseed rape plants. Experiments on both primary and secondary splash events were done using a rain tower, and the resulting ballistic trajectories of droplets splashing from different plant parts were estimated.

4.2 Materials and Methods

The incorporation of *P. brassicae* conidia into droplets and their removal from oilseed rape plants were investigated in experiments using simulated rain. Experiments were done on primary and secondary splash. Weighted mean trajectories of splash droplets were estimated in experiments using single raindrops.

4.2.1 Rain simulation

All experiments were done in the rain tower at the Institute of Arable Crop Research, Harpenden, UK (Fitt et al., 1986^a). The rain tower was 11 m high, with a 1 m square cross section, allowing raindrops to reach close to their terminal velocity at the base. The rain simulator consisted of a 1 m square frame containing ten 1 m long plastic tubes, 20 cm in diameter, in parallel rows. Detachable hypodermic needles were inserted 11.5

cm apart along the length of each tube, giving an array of up to 80 drop sources. Drop size depended on the gauge of the needles. Experiments were done using two needle sizes to produce drops with diameters of approximately 2.4 and 2.9 mm. The generator was fed with dionised water using a peristaltic pump. Drop diameter was estimated by measuring the mass of 200 drops from each needle. The intensity of the simulated rain showers was measured by moving a 195 mm diameter collector through random positions under the simulator and measuring the mass of water collected over a known time period. The intensity of showers of 2.4 mm diameter drops was 1.33 mm h^{-1} and the intensity of showers of 2.9 mm diameter drops was 1.05 mm h^{-1} . All experiments were done using glasshouse grown oilseed rape plants (cv Bristol or cv Rebel).

4.2.2 Primary splash

The removal of conidia from light leaf spot lesions by primary splash was assessed using infected sporulating leaves of 7-8 week old oilseed rape plants. Four week old plants were inoculated by spraying them with a suspension of *P. brassicae* conidia using a spinning disc atomiser (40 ml with c. 10^6 conidia ml^{-1} at 8 ml min^{-1}). They were placed in polyethylene bags, to maintain 100% humidity, and kept at 15°C for two days. The plants were then maintained at 15°C until sporulation of *P. brassicae* occurred after about two weeks.

Three leaves of similar size showing similar symptoms were removed from the infected plants. The surface area of individual leaves was measured by placing each leaf on a sheet of squared paper and counting the number of squares covered. One leaf was exposed at the base of the rain tower to simulated rain for 15 min; a second leaf was exposed for 25 min, the third leaf (control) was kept in the rain tower but not exposed to rain. It was assumed that there was a similar number of conidia per unit area on the surfaces of the control leaf and the two exposed leaves. After exposure to rain both the exposed and control leaves were cut into strips, which were placed in separate universal bottles and washed with first 20 ml and then a further 10 ml of distilled water. The universal bottles were agitated to remove conidia from the leaf strips. The concentration of conidia in the washing water was estimated using a haemocytometer slide. The number of conidia per unit area of control and exposed leaves was estimated from the total leaf area and the number of conidia in the washings. The number of conidia removed by the rain showers was estimated by subtracting the number of conidia per unit area remaining on the exposed leaves from the number of conidia per unit area on the control leaf.

4.2.3 Secondary splash

For the secondary splash experiments, healthy oilseed rape plants were sprayed with a conidial suspension using an atomiser (c. 10^6 conidia per ml). Experiments were done using young leaves (7-8 week old plants). For the experiments three similar leaves were

Table 4.1: Calculated mean percentage conidia removed from leaves in the primary and secondary splash experiments when different rain showers were applied. Numbers in parentheses represent standard deviations.

Drop size (mm)	Rain duration (min)	Number of experiments	Mean % conidia removed Primary splash
2.4	15	2	24 (4)
2.4	25	1	63
2.9	15	6	33 (25)
2.9	25	1	83
			Secondary splash
2.4	15	2	65 (3)
2.9	15	4	51 (24)

tower, at an angle similar to that observed in oilseed rape crops, and exposed to one raindrop of 2.4 or 2.9 mm diameter. A drop of colored ink was pipetted on the drop impact site to act as a tracer for the splash droplets. Splash droplets were collected on a 110 × 110 cm filter paper (Whatman Ltd.) placed at the base of the rain tower, 15, 30 or 50 cm below the splash target (Figure 4.1). The horizontal distances travelled by splash droplets were measured from the traces left on the filter paper. A different colour ink drop was used within each experiment when the target was adjusted to another vertical distance from the base of the rain tower. Leaf targets were adjusted so that the impact points were either within the distal third of the leaf (leaf tip) or within the proximal third of the leaf (leaf base). Similar experiments were done using Petri dishes containing glasshouse compost as the target. In these experiments splash droplets were collected on water sensitive paper (Sheriff Cropcare Ltd., Royston, Herts, UK), and dispersal distances were estimated from the trace of the splash droplets.

4.3 Results and interpretation

The results of the primary and secondary splash experiments are presented in Table 4.1. More conidia were removed from leaves when rain duration increased from 15 to 25 min. For 15 min rain exposures conidia appeared more easily released during secondary splash events than from the original lesion. For example, the proportion of conidia removed by secondary splash was about twice that for primary splash. For primary splash, the larger drops tended to be more effective in removing conidia than the smaller ones. However, for secondary splash both sizes of drop appeared to be equally effective. Thus, in a primary splash event, larger raindrops may be needed for the final detachment of

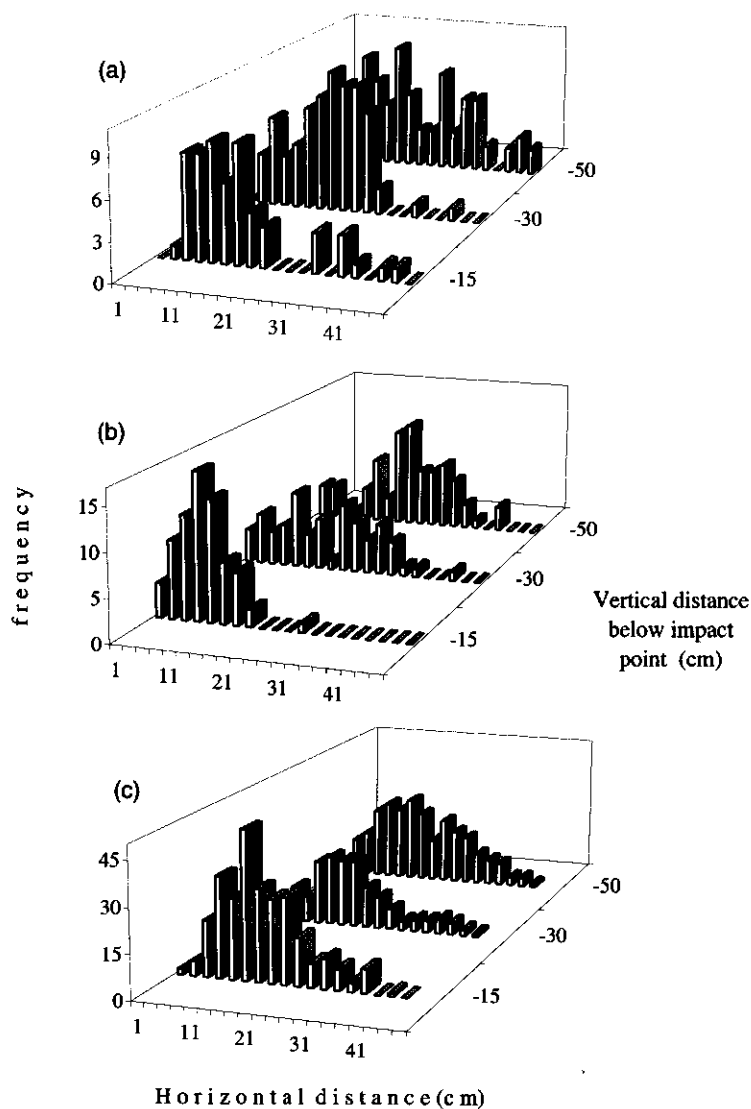


Figure 4.2: Frequency distributions of horizontal distance travelled by droplets splashed from a leaf tip (a), flower (b) or pod (c) and collected 15, 30 and 50 cm below the impact point. Diameter of the impacting drop was 2.4 mm.

Table 4.2: Horizontal distances (weighted mean) travelled by droplets splashed from the base of a leaf, a leaf tip, flower, pod or the ground. Standard deviations of the distributions are given in parenthesis.

Plant part	Drop size (mm)	Weighted mean horizontal distance (cm)		
		y=15	y=30	y=50
Leaf base	2.4	20.5 (10.6)	26.4 (10.5)	26.7 (11.6)
Leaf base ^a	2.4	12.3 (6.6)	14.4 (6.9)	17.0 (7.3)
Leaf base	2.9	23.7 (8.9)	21.8 (10.0)	29.4 (13.8)
Leaf base	2.9	10.5 (5.2)	10.5 (6.7)	18.4 (8.7)
Leaf base ^a	2.9	7.7 (4.9)	13.3 (8.0)	11.0 (6.0)
Leaf tip	2.4	16.2 (10.2)	17.9 (8.2)	23.6 (11.4)
Leaf tip ^a	2.4	14.0 (8.4)	15.1 (7.2)	17.7 (9.2)
Leaf tip ^a	2.9	14.7 (7.2)	18.6 (9.6)	21.1 (10.6)
Leaf tip	2.9	16.6 (7.4)	15.7 (8.7)	18.6 (9.0)
Leaf tip	2.9	9.3 (5.6)	17.7 (9.9)	14.8 (8.4)
Flower ^a	2.4	9.4 (4.9)	17.1 (10.0)	21.0 (9.3)
Flower ^a	2.9	8.8 (4.5)	12.5 (8.8)	15.3 (8.0)
Pod	2.4	16.6 (7.7)	18.0 (8.7)	18.5 (9.4)
Pod ^a	2.4	13.5 (6.9)	14.1 (7.9)	18.3 (9.1)
Pod	2.4	16.6 (8.1)	9.5 (4.8)	17.1 (9.0)
Pod ^a	2.9	13.9 (7.1)	19.6 (10.9)	20.3 (8.9)
Ground ^a	2.4	21.5 (9.3)	21.6 (9.3)	24.5 (10.7)
Ground ^a	2.9	21.8 (10.5)	20.7 (9.1)	23.8 (9.7)

^aData shown in Figures 4.3 and 4.4

conidia from the acervuli where they are produced on when the leaf becomes wet. The smaller raindrops may support spread of spores from the water film on healthy plant tissue in secondary splash events.

The frequency distributions for horizontal distance travelled by splash droplets collected 15, 30 and 50 cm below a leaf tip, flower and pod target are shown in Figure 4.2. The distributions were typical of those found in the other experiments.

The horizontal distances (weighted mean) for droplets splashing from the leaf base, leaf tip, flower, pod and the ground are presented in Table 4.2 and an example from each target type is shown in Figures 4.3 and 4.4. The mean distances travelled by the splash droplets tended to increase with the height of the target above the collection surface. The variability between replicate experiments tended to be relatively large,

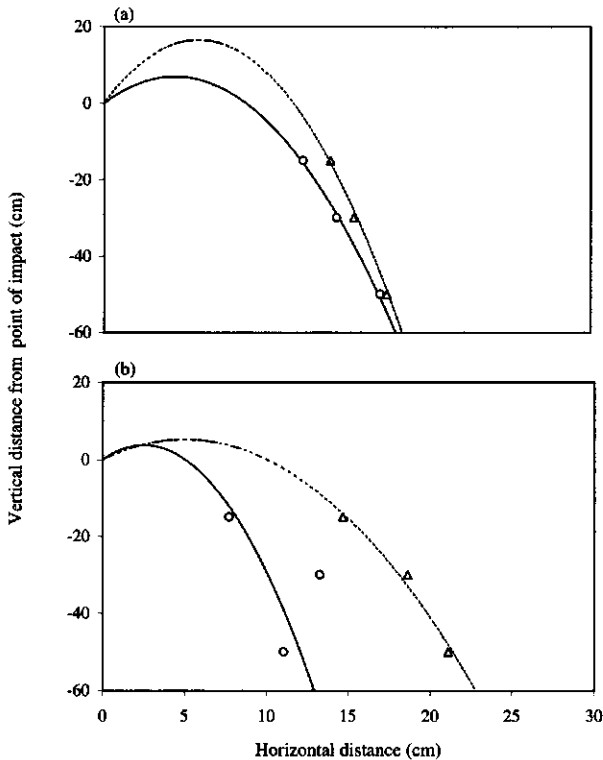


Figure 4.3: Horizontal dispersal distances (weighted mean), measured 15, 30 and 50 cm below leaf targets for (a) 2.4 and (b) 2.9 mm impacting drops. ○ splash from base of leaf; △ splash from tip of leaf. Parabolic trajectories fitted to the measured distances are shown as solid (base) and broken (tip) lines.

especially for splashing from leaves. For example, the mean distance travelled by droplets splashing from the base of leaves ranged between 11 and 29 cm for 2.9 mm impact drops (3 replicates). There were no clear consistent differences in dispersal patterns for the different targets. For all targets, dispersal distances ranged between about 8 and 24 cm, 10 and 26 cm and 11 and 29 cm for droplets collected at 15, 30 and 50 cm below the target, respectively.

An “average” trajectory for splash droplets was defined as that trajectory that passed through the point of impact and the weighted mean distances calculated from the traces of ink on the filters at the three distances below the target (x_1 , x_2 , and x_3). The trajectories of individual splash droplets depend on their size, mass and initial velocity and on the local air flow. In still air, trajectories can be calculated, taking account of

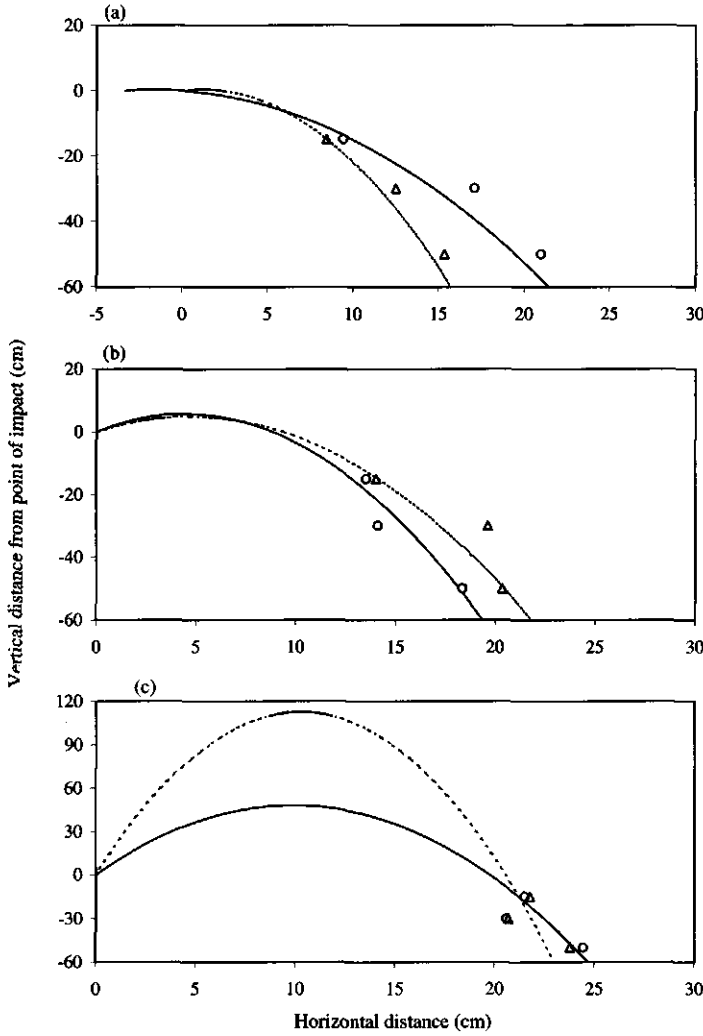


Figure 4.4: Horizontal dispersal distances (weighted mean), measured 15, 30 and 50 cm below (a) flowers, (b) pods and (c) soil. \circ 2.4 mm impacting drop; \triangle 2.9 mm impacting drop. Parabolic trajectories fitted to the measured distances are shown as solid (2.4 mm drops) and broken (2.9 mm drops) lines.

effects of air resistance, using classical Newtonian dynamics (Macdonald and McCartney, 1987; Allan, 1988). The trajectories of splash droplets, in still air, are roughly parabolic in shape, although drag forces tend to truncate the shape at large distances. The effect is more marked for small drops, when drag forces are proportionally more important. Because of the non-linear relationships between drag forces, velocity and droplet size, calculation of trajectories is not trivial and no simple analytical solutions exist. If drag forces are ignored, the solution of a particle trajectory, in still air, is a simple parabola, whose slope depends only on the droplet mass and initial velocity. For splash droplets of the size normally associated with spore transport ($\sim 200 \mu\text{m}$ or greater, Fitt *et al.*, 1989) the above simplifying assumption is probably adequate when incorporating droplet transport into disease progress models. Therefore, a parabolic equation of the form

$$x = (-b - \sqrt{(b^2 + 4ay)})/2a \quad (4.1)$$

was fitted to the "average" trajectories derived from the splash experiments. The parameters, a and b , of the fitted parabolas are given in Table 4.3. The maximum droplet height predicted by each fitted equation is given in Table 4.3. Generally, the measured "average" trajectory was well fitted by the parabolic equation.

For the 2.4 mm drops impacting on a plant surface, the highest vertical distance reached by splash droplets predicted by the fitted parabola was 56.7 cm for a pod (Table 4.3) and the lowest was 0.3 cm for a flower (Table 4.3 and Figure 4.4a). For 2.9 mm drops, maximum heights predicted ranged between 0.4 to 39.8 cm for splashing from a flower and the tip of a leaf, respectively (Table 4.3 and Figure 4.4a). The highest vertical distances predicted were 112 and 302 cm from a 2.9 mm drop impacting on soil (Figure 4.4c) and a pod (Table 4.3) respectively. The two trajectories for splash on flowers suggested that droplets fell almost directly to the ground, possibly because the flowers were deflected by the impacting raindrop, so that most of the droplets were directed downwards.

4.4 Discussion

The results of this study clearly demonstrate that conidia of *P. brassicae* have the potential to be further dispersed by the action of secondary splash after an initial primary splash from infected plant tissue. The results suggest that splash may be at least as efficient at removing deposited conidia from leaves as from sporulating *P. brassicae* infections. However, it is not known how long a conidium deposited on a wet leaf remains available for redispersal by rain-splash. Our experiments suggest that secondary splash can take place at least 30 min after primary dispersal. There have been few other studies on secondary splash dispersal of plant pathogenic fungi. Madden *et al.* (1996) found that between 46 and 83% of *Colletotrichum acutatum* spores were removed from infected

Table 4.3: Estimates of the parameters of the parabolic equation fitted to the measured "average" droplet trajectories for 2.4 and 2.9 mm drops impacting onto different plant structures. Numbers in parenthesis represent asymptotic standard errors.

Plant part	Drop diameter (mm)	Parameter estimates		Max height (cm)
		a (cm)	b (cm)	
Leaf base	2.4	-0.18 (0.058)	3.09 (1.409)	13.45
Leaf base ^a	2.4	-0.36 (0.088)	3.15 (1.287)	6.91
Leaf base	2.9	-0.20 (0.084)	3.74 (2.063)	17.12
Leaf base	2.9	-0.08 (0.030)	-1.29 (0.519)	5.18
Leaf base ^a	2.9	-0.59 (0.229)	2.99 (2.181)	3.80
Leaf tip	2.4	-0.16 (0.053)	1.46 (1.042)	3.35
Leaf tip ^a	2.4	-0.48 (0.100)	5.66 (1.516)	16.50
Leaf tip ^a	2.9	-0.21 (0.072)	2.09 (1.295)	5.30
Leaf tip	2.9	-0.75 (0.194)	10.92 (3.337)	39.82
Leaf tip	2.9	-0.75 (0.227)	8.63 (3.366)	24.66
Flower ^a	2.4	-0.11 (0.030)	-0.38 (0.512)	0.32
Flower ^a	2.9	-0.29 (0.088)	0.68 (1.075)	0.40
Pod	2.4	-0.92 (0.383)	14.41 (6.735)	56.65
Pod ^a	2.4	-0.29 (0.078)	2.59 (1.310)	5.72
Pod	2.9	-4.70 (4.994)	75.33 (81.923)	302.12
Pod ^a	2.9	-0.22 (0.049)	2.08 (0.908)	4.86
Ground ^a	2.4	-0.49 (0.332)	9.74 (7.383)	48.13
Ground ^a	2.9	-1.07 (1.010)	21.91 (21.906)	112.43

^aData shown in Figures 4.3 and 4.4

strawberry fruit by 16 min exposures to simulated rain (primary splash) compared with 22-59% removal from healthy fruit sprayed with spores (secondary splash). Ntahimpera *et al.* (1999) found similar proportions of spores of *Colletotrichum* spp. removal from infected and healthy strawberry fruit when exposed to similar rain showers of different intensities. The proportion of *P. brassicae* conidia removed by secondary and primary splash, are comparable to these found in the studies of *Colletotrichum* spore dispersal. This suggests that secondary splash dispersal may be more important than previously recognized for a number of plant pathogens.

There have been few direct studies on trajectories of droplets splashing from plants (Reynolds *et al.*, 1987; Macdonald and McCartney, 1988; Yang *et al.*, 1991). Modelling splash droplet trajectories can be complex and requires knowledge of both the distribution of initial velocities and droplet size distributions. Thus, to incorporate splash

dispersal into disease spread models within canopies a simplified approach to dispersal modelling is required. The results of these splash experiments suggest that, for practical modelling purposes, "average" trajectories of droplets splashed from oilseed rape plant parts may be approximated by simple parabolic equations. The parabolic equations, fitted to observed dispersal distances, allowed an estimation of the vertical height reached by splash droplets. Values for leaves were comparable with the range of values (between ~ 12 and 20 cm) reported by Walklate *et al.* (1989) for splash from oilseed rape leaves. However, these results suggest that the structure of plant parts may strongly influence splash droplet trajectories. Estimated maximum heights for splash from flowers were smaller than those from leaves or pods, probably because the flowers were moved by raindrops, deflecting the splash droplets downwards. Macdonald and McCartney (1988) found that a substantial number of splash droplets were directed downwards from splash on bean (*Vicia faba*) leaves, probably due to leaf flexing during impact.

Rain-splash is an important agent in the dissemination of plant pathogen propagules. Knowledge of the process is needed to understand the development of epidemics in plant canopies. Secondary splash may have the potential to disperse fungal spores further after their initial dispersal from disease lesions. Further work is needed to quantify the effect of secondary splash in spore dispersal and to determine if it is necessary to separate primary from secondary splash in models of disease spread. Although the mechanisms of splash dispersal are complex, it may be possible to incorporate splash dispersal trajectories into disease simulation models, using simple parabolic models, once the relationships between model parameters and rainfall and crop structure are known.

Acknowledgements

The investigations for project 05-39-181 were supported by the Life Sciences Foundation (SLW), which is subsidized by the Netherlands Organization for Scientific Research (NWO). IACR-Rothamsted is supported by a grant from the Biotechnology and Biological Research Council, UK. We thank M.J. Jeger for helpful advice and T. Gilles and J. Steed for help with the experimental work.

Chapter 5

Simulation of vertical spread of plant diseases in a crop canopy by stem extension and splash dispersal.

A. Pielaat F. van den Bosch B.D.L. Fitt M.J. Jeger¹

Abstract

Upward displacement of lesions by stem extension and dispersal of fungal conidia by rain-splash are mechanisms contributing to within-crop disease spread. These mechanisms were incorporated into a model, using the interaction between winter oilseed rape and the light leaf spot pathogen (*Pyrenopeziza brassicae*) as an example. Development of leaves, flowers and pods over the period from the start of stem extension to pod ripening was simulated. In the model, as new plant parts developed from the apex during stem extension, infections on plant initials were spread to the upper canopy by internode growth. In addition, conidia produced by the pathogen were dispersed in the canopy by rain-splash and produced lesions at new sites. Vertical disease spread was simulated with a number of different disease distributions at the start of stem extension and different crop structures and rain durations. Results showed that stem extension was an important factor influencing vertical disease spread in the model oilseed rape crop. Rain events

¹Ecological Modelling (submitted)

contributed to the splash dispersal of conidia to the plant apex and resulting infections were directed vertically by internode growth. Periods with frequent rain events in a dense crop canopy (LAI constant with height) were most favorable for disease progress. The upward spread of light leaf spot on winter oilseed rape in experiments at the Institute of Arable Crop Research, Harpenden, UK was similar to that predicted by the model.

5.1 Introduction

Disease spread to upper leaves and seed-bearing organs (pods for oilseed rape, ears for cereals) is important in the development of damaging epidemics in winter-sown arable crops in Europe, but these phenomena are difficult to model. Epidemics of many plant diseases caused by ascomycete fungal pathogens are initiated by wind-borne ascospores transported into the crop in autumn (Inman et al., 1999). In the crop, disease epidemics spread horizontally during the vegetative growth phase, whilst crops remain prostrate (in autumn and winter in Europe) and subsequently spread up plants when stem extension occurs in spring. Initial ascospore infection can however also occur in spring. Subsequent spread is often by rain-splash dispersed conidia. Disease spread by splash droplets has been reported for many pathogens (Evenhuis et al., 1997; Fatemi and Fitt, 1983; Fitt et al., 1989; Madden, 1992; Soleimani et al., 1996). Vertical spread of splash-dispersed plant pathogens plays a particularly important role in epidemic progress (Inman and Fitt, 1992; Lovell et al., 1997; Shaw, 1987). Polycyclic diseases for which secondary spread is mainly by splash dispersal include light leaf spot (*Pyrenopeziza brassicae*) and white leaf spot (*Mycosphaerella capsellae*) on winter oilseed rape, septoria leaf blotch (*Mycosphaerella graminicola*) and glume blotch (*Stagnospora nodorum*) on winter wheat and leaf blotch (*Rhynchosporium secalis*) on winter barley.

In many experiments, the influence of rainfall conditions on spatial spread of disease have been studied, both in arable and horticultural crops (Fitt et al., 1986; Madden, 1997; Madden et al., 1996; Ntahimpera et al., 1997; Yang and Madden, 1993). Inman and Fitt (1992), for example, showed how vertical movement of conidia by rain-splash contributes to progress of white leaf spot epidemics on winter oilseed rape. Results from field and laboratory experiments have been used to construct models predicting the horizontal spread of pathogens exposed to simulated rain events (Boudreau and Madden, 1995; Madden et al., 1996; Pielaat and van den Bosch, 1998; Pielaat et al., 1998). Much work has been done to produce empirical models to describe horizontal disease spread but little work has been done to develop mechanistic models to describe vertical disease spread. Exponential or power law equations and diffusion models have frequently been used to describe deposition gradient data sets. However, such descriptive models give only a qualitative view of the spatial disease spread for a particular experiment (Fitt et al., 1987; Fitt and McCartney, 1986; McCartney and Fitt, 1986). These descriptive models cannot be used to predict the spatial spread of the disease in different situations.

For this purpose, physical models which incorporate the mechanisms by which plant pathogens are splashed have been developed. The effects of environmental and biological factors on disease spread can be studied using such mechanistic models (Pielaat and van den Bosch, 1998; Pielaat et al., 1998; Yang et al., 1991).

Several diseases of arable crops can be spread vertically in the crop canopy by rain-splash, but the relative importance of splash dispersal and upward spread of infected tissue by stem extension is still unclear. There is a need for generic models to describe mechanisms of vertical disease spread so that, at the time when control spray decisions are needed, predictions can be made about disease incidence and severity on upper leaves. Since *P. brassicae* can infect meristematic tissue, stem extension may be particularly important in the spread of light leaf spot on winter oilseed rape.

This paper describes the development of a simulation model for upward spread of diseases in winter-sown arable crops by stem extension and by splash dispersal, using light leaf spot on winter oilseed rape as a model system.

5.2 The model

The model simulated upward spread of light leaf spot in spring during the period after the start of stem extension (growth stage 2.4) of winter oilseed rape until June (growth stage 6.1). Su et al. (1998) showed that yield loss from light leaf spot could be predicted well by assessing light leaf spot incidence during this period, i.e. early flowering, growth stage 3.3, Sylvester-Bradley (1985). Plant growth was modelled using published data on surface area development in oilseed rape. Conidia were dispersed to other plant parts by rain. Transition probabilities determined where a conidium was deposited when splashed by rain from a particular site in the crop. A fraction of the conidia was transported up the plant as new plant parts (leaf, flower or pod) emerged.

The model described how light leaf spot moves up the crop during the period of stem extension in Europe (assumed to start on March 17), until late flowering (assumed to end on June 5). The influence of the frequency of rain events and of rain duration on disease development was evaluated by simulating contrasting seasons when this period was dry or wet. In addition, separate simulations were applied to produce three virtual crops with different crop density distributions to evaluate the influence of leaf surface area distribution on disease development.

5.2.1 Disease description

Light leaf spot (*Pyrenopeziza brassicae*) is an important disease of winter oilseed rape (*Brassica napus*) in northern Europe (Fitt et al., 1998; Jeffery et al., 1989; Jeffery et al., 1994). This polycyclic disease produces lesions on the different plant tissues (i.e. leaves, stems, flowers and pods) during the season (Fitt et al., 1998^a). Each year, *P.*

brassicae has a sexual cycle followed by several asexual cycles; each type of cycle has different infectious units. Ascospores are produced on crop residues during the sexual cycle at the end of the growing season (late summer) and are dispersed by wind. They have the potential to be dispersed over relatively large distances and are therefore likely to cause primary infection in new crops sown in the autumn. After primary infection, the asexually produced conidia are the principal dispersal units in consecutive epidemic cycles during the growing season. These conidia are dispersed by rain-splash to infect new leaves (a latent infection). After a latent period, *P. brassicae* produces new conidia in a sporulating infection, and a new cycle of splash dispersal proceeds during the next rain event.

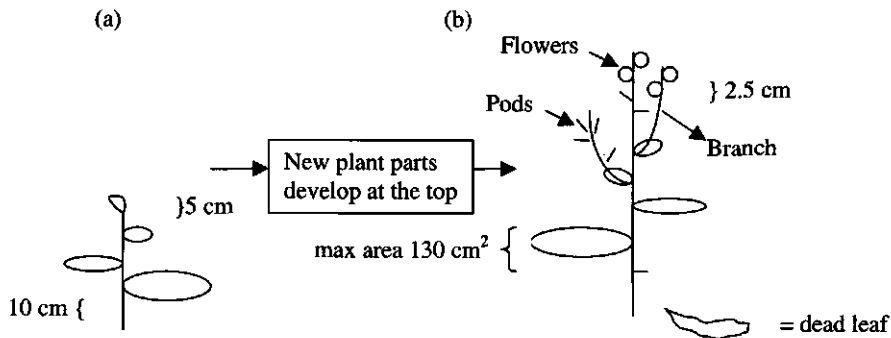


Figure 5.1: Phenology of the model winter oilseed rape plant (a) at early stem extension (growth stage 3.3) and (b) at late flowering (growth stage 5.2) (Sylvester-Bradley, 1985). The model assumes that: the first leaf is 10 cm above the ground, subsequent leaves develop with distances of 5 cm between them and flowers and pods develop 2.5 cm apart; the maximum surface area a leaf can reach before abscission is 130 cm².

5.2.2 Plant growth during stem extension

Plant morphology during the period of stem extension was modelled using phenological information about winter oilseed rape (Habekotté, 1996; Inman and Fitt, 1992; Mendham and Salisbury, 1995; Morrison et al., 1992; Tayo and Morgan, 1975). This model assumed that the process of winter oilseed rape growth during stem extension is not affected by light leaf spot. When light leaf spot epidemics are severe, the disease will affect crop growth and thus cause large yield losses. However, farmers generally apply fungicides to protect their crops from such severe epidemics. Nevertheless, light leaf spot epidemics which do not affect the process of stem extension *per se* may still cause significant yield losses through damage to leaves, stems and pods.

In the model system, stem extension begins in March and the disease is spread in the crop by two mechanisms (Remmelzwaal and Habekotté, 1986). The changes in phenology of an oilseed rape plant were therefore modelled from March 17, which is referred to as "the start of the period of stem extension". At the start of this period, the model plant had 10 leaves; the first and lowest leaf was located 10 cm above the ground and each successive leaf was produced 5 cm above the previous one (Mendham and Salisbury, 1995) (Figure 5.1a). The distance between successive leaves was assumed to be constant during the period of growth. New plant parts (leaves, flowers and pods) were formed only at the top of the plant as it grows. During rain events, conidia from existing lesions were splash dispersed to other sites in the crop. If the surface areas of all plant parts are known, then probabilities can be calculated for transition of conidia by splash from a particular location in the crop to be deposited on to the ground, a leaf, a flower or a pod.

In the model, individual leaves reached their maximum surface area by mid-May; thereafter flowers and pods were produced (Mendham and Salisbury, 1995, Fig. 2.6). The first ten leaves already present before the period of rapid stem extension were allowed to reach a maximum surface area of 130 cm² before they abscised. The maximum surface area of leaves which were formed after mid-March declined with time of appearance, because leaf expansion was assumed to stop by mid-May (Figure 5.1b). During the period of stem extension, the model plant formed five branches on which flowers developed, in addition to those on the main stem (Mendham and Salisbury, 1995 (Fig. 2.11); Tayo and Morgan, 1975 (Fig.5)). The first flower appeared at the beginning of April and flowers were assumed to have a constant surface area of 1 cm² from the day of their appearance. In the model, the first pod developed five days after the start of flowering and pods grew to a maximum surface area of 2 cm² (Mendham and Salisbury, 1995 (Fig.2.10); Tayo and Morgan, 1975 (Table 3)). Flowers were allowed to develop 2.5 cm apart (Figure 5.1b) but more than one flower could develop at the same height and only a proportion of the flowers produced pods (Mendham and Salisbury, 1995 (Fig. 2.11); Tayo and Morgan, 1975 (Fig.5)). The changes in surface area through development and abscission of leaves, flowers and pods of the model winter oilseed rape plant from March 17 to July 20 are shown in Figure 5.2. Disease spread by the two mechanisms, and with that the model simulations, stopped on June 5 (day 81 in Figure 5.2). By the end of June leaf expansion stopped and the plant progressively senesced until the end of July when the crop was harvested.

The crop was divided into 80 height-categories, each of 2.5 cm, to give a total height of 200 cm (Habekotté, 1996). In the crop, a lesion could be positioned on a leaf, a flower or a pod growing on the the main stem or the branches. Depending on plant age, each height category contained specific values for leaf, flower and pod surface areas. In the model, it was assumed that branches and stems did not have lesions, since light leaf spot lesions on stems are generally superficial and not damaging (Fitt et al., 1998^a).

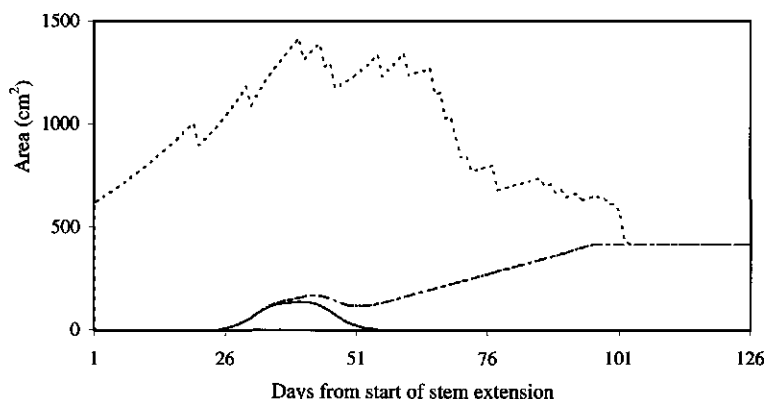


Figure 5.2: Cumulated surface area development with time for all leaves (dotted line), flowers (solid line) and pods (dashed line) of the model winter oilseed rape plant during growth from 17 March (growth day 1) until 20 July (growth day 126). Flowers exist from day 25 until 55; pods from day 30 until 126.

5.2.3 Disease dynamics

After each rain event, a fraction of the conidia dispersed by rain-splash reached susceptible tissues and germinated to cause latent infections. Assuming an exponential distribution with a mean latent period of 17 days for *P. brassicae* (Figuroa et al., 1995, Table 2), the model estimated that each day a fraction $\frac{1}{17}$ of latent infections became sporulating lesions. Sporulating lesions of *P. brassicae* were allowed to survive throughout the period of stem extension and to produce successive generations of conidia until tissues senesced. In the model, sporulating tissue on each leaf had a surface area of 0.36cm^2 and did not expand (Figuroa et al. (1995) observed a maximum of 28 lesions per 10cm^2 leaf surface area at 15°C). Each sporulating lesion was assumed to produce a fixed number of conidia as soon as it started raining. During a rain event, conidia from these lesions were dispersed in the crop. They could also fall to the ground and be resplashed back into the crop. Conidia still on the ground after the rain event were assumed to be removed from the epidemic process (Pielaat et al., 1998). Lesions on leaves that abscised and fell down to the ground were also removed. A general summary of the model, including the parameters, is given in Figure 5.3. The model included the following sub-routines:

Disease initiation and rain events. The numbers of latent infections and sporulating lesions for each of the height categories on a plant at the start of stem extension

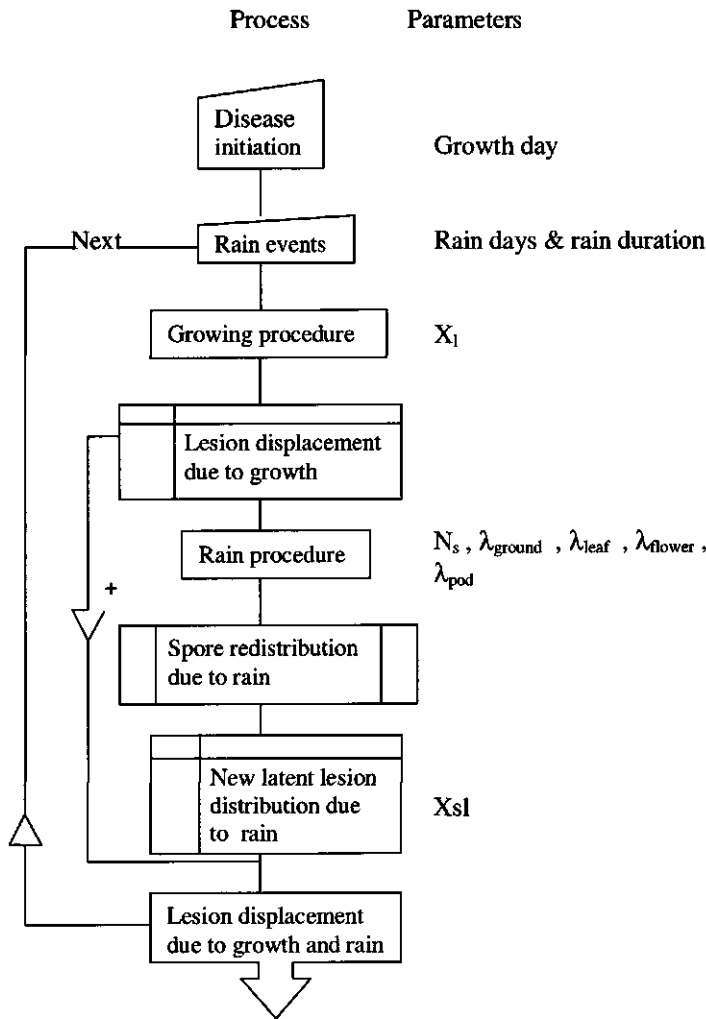


Figure 5.3: The model; processes and accompanying parameters required to simulate vertical progress of light leaf spot by rain-splash events and stem extension of a winter oilseed rape crop. (X_i is the fraction of latent infections that become sporulating per day; N_s is the number of spores produced by a sporulating infection; λ_{ground} , λ_{leaf} , λ_{flower} and λ_{pod} are the probabilities that a spore will be splashed from the different targets; X_{sl} is the fraction of spores that become latent infections after the rain event.

formed the initial conditions for the model. After the initial distribution of lesions on the leaves had been specified, subsequent disease progress was simulated throughout the period of stem extension. As vertical disease progress is influenced by both plant extension and rain events, the next input variables for the model were the days on which it rained and the rain duration.

Lesion displacement due to growth. The stem extension procedure simulated upward displacement of lesions prior to the next rain event. In this procedure, the next day was compared with the current day; if on the next day a leaf abscised and fell to the ground, lesions were removed from the epidemic. If a flower abscised, lesions were either removed or, if a pod developed, transmitted to the pod. Pods remained until the end of the period. New leaves are initiated at the apex (growing point) of the plant well before stem extension and leaf development (Mendham and Salisbury, 1995). In the model, conidia deposited by rain-splash on to the apex were distributed between new leaves in proportion to leaf number and size. For the displacement of infectious units to new flowers, the same procedure was applied. As branches are initiated at the apex near a leaf, the first flower on a branch received lesions in proportion to the surface area of the leaf that it developed from. Each pod received all lesions from the flower from which it developed. In addition, each day in each height category (H) on the plant, a fraction $X_L (= \frac{1}{17})$ of latent infections (L) became sporulating lesions (S):

$$\begin{aligned} S_{H,t+1} &\rightarrow X_L L_{H,t} \\ L_{H,t+1} &\rightarrow (1-X_L) L_{H,t} \end{aligned}$$

Upward displacement of latent infections and sporulating lesions by stem extension proceeded until the first rain event, when vertical displacement by splash dispersal commenced; subsequently vertical displacement by both mechanisms was included.

Rain Procedure. In the model, sporulating lesions released conidia when rain started. Each sporulating lesion was assumed to contain a constant number of conidia (N_s) per rain event. The number of conidia at height h during the rain event was determined by the number of conidia splashed from all other positions n to h , and the number of conidia splashed away from height h .

The number of conidia at different heights was calculated using a series of differential equations. $S_h(t)$ represented the number of conidia at height h at time t after the start of the rain event (i.e. $h=0$ represented the ground and $h=\max$ represented the maximum height of the plant at that crop growth stage). α_{nh} was the probability per unit time that a conidium was splashed from height n to h and λ_h was the probability per unit time that it was splashed away from height h . The series of differential equations was then:

$$\begin{aligned}
\frac{dS_0}{dt} &= \alpha_{00}S_0 + \alpha_{10}S_1 + \dots + \alpha_{max0}S_{max} - \lambda_0S_0 \\
\frac{dS_1}{dt} &= \alpha_{01}S_0 + \alpha_{11}S_1 + \dots + \alpha_{max1}S_{max} - \lambda_1S_1 \\
&\vdots \\
\frac{dS_{max}}{dt} &= \alpha_{0max}S_0 + \alpha_{1max}S_1 + \dots + \alpha_{maxmax}S_{max} - \lambda_{max}S_{max}.
\end{aligned}$$

In these equations, α_{nh} was the probability that a conidium was splashed from height n (λ_n) multiplied by the probability of deposition at height h when splashed from n (P_{nh}) (i.e. $\alpha_{nh} = P_{nh} \cdot \lambda_n$). The probability that a conidium was splashed from height n (λ_n) depended on the number of leaf, flower and pod layers that the incoming raindrop had to pass through to reach height n . Thus λ_n was calculated as:

$$\lambda_n = L_n \cdot B_n, \quad (5.1)$$

where L_n is λ_n for a conidium in direct contact with open air and B_n is the probability that a raindrop penetrated the crop canopy down to height n . The parameter L_n was determined experimentally for conidia splashing from the ground, from a leaf, from a flower or from a pod (Pielaat, Marshall, McCartney, van den Bosch and Fitt, chapter 4). B_n was calculated from the vertical canopy surface area profile. The term Plant Area Index (PAI) was used to represent the total surface area (cm^2) of leaves, flowers and pods in a given height category per cm^2 of ground. To penetrate the crop down to height n , an incoming raindrop had to pass through the upper height category (of 2.5 cm) and the height category below that and so on until it reached height n . The probability that a raindrop passed down to height category h was proportional to the PAI of height h :

$$B_n = \left(\prod_{h=max}^{n+1} \text{pass height } h \right) \cdot \text{intercepted by height } n.$$

The probability that a raindrop penetrated the crop down to height n was then calculated:

$$B_n = e^{-\theta PAI_{h=max}} \cdot e^{-\theta PAI_{h=max-1}} \cdot \dots \cdot e^{-\theta PAI_{n+1}} \cdot (1 - e^{-\theta PAI_n}), \quad (5.2)$$

where $e^{-\theta PAI_h}$ was the probability that the raindrop was not intercepted at height h (Pielaat, van den Bosch, de Gee and van der Wal, chapter 6). In (5.2), θ was a parameter depending upon the surface area distribution in the crop. For the present purposes θ was set to 1.

Similarly, P_{nh} , the transition probability that a conidium was splashed from height n to height h , was calculated from the PAI. Each transition probability was calculated by examining all possible routes through which a conidium could reach height h when it was splashed from height n . Splash heights for droplets splashing from the ground, from a leaf, from a flower or from a pod were determined experimentally (Pielaat, Marshall, McCartney, van den Bosch and Fitt, chapter 4) to produce a frequency distribution of maximum splash heights for droplets splashing from the different sites in the crop. With these results, all transition probabilities were calculated, including the frequency with which a droplet followed a specific trajectory to make a specific transition. For example, the probability that a conidium, splashed from a leaf at height $n=1$ returned to height $h=1$, assuming that a droplet splashed to a maximum height of 3 cm (passing a maximum of one height category of 2.5 cm before falling down again) half the times and splashed to a maximum height of 5 cm (passing two height categories) half the times, was:

$$P_{11} = [\text{stay in height 1} + \left(\prod_n^{h+1} \text{pass height } n \right) \cdot \text{intercepted by height } n] \cdot 0.5 \\ + [\text{stay in height 1} + \left(\prod_n^{h+2} \text{pass height } n \right) \left(\prod_{h+1}^{n+1} \text{pass height } n \right) \cdot \text{intercepted by height } n] \cdot 0.5$$

Thus resulted in:

$$P_{11} = [(1 - e^{-\frac{1}{2}\theta PAI_1}) + e^{-\frac{1}{2}\theta PAI_1} \cdot e^{-\theta PAI_2} \cdot (1 - e^{-\theta PAI_1})] \cdot 0.5 + \\ [(1 - e^{-\frac{1}{2}\theta PAI_1}) + e^{-\frac{1}{2}\theta PAI_1} \cdot e^{-\theta PAI_2} \cdot e^{-\theta PAI_3} \cdot e^{-\theta PAI_2} \cdot (1 - e^{-\theta PAI_1})] \cdot 0.5. \quad (5.3)$$

Since a conidium splashed, on average, from the central part of a height category, the PAI of height 1 (in this case) was multiplied by 0.5.

After calculating all parameters for the series of differential equations, they were solved numerically for the time interval $t=0$ to $t=T$, the duration of the rain event. However, an additional complicating factor was that the probability that a conidium splashed from height n to height h depended on the part of the crop from which a conidium splashed. As there were four parts available (i.e. the ground (g), leaves (l), flowers (f) and pods (p)), each parameter α_{nh} incorporated four different values. The

probability that a conidium splashed from a site at height n was given by λ_n . Each parameter α_{nh} of the series of differential equations was therefore subdivided:

$$\alpha_{nh} = P_{nh} \cdot B_n \cdot \lambda_g \quad \text{for } n = 0 \quad (5.4)$$

$$\alpha_{nh} = P_{nh} \cdot B_n \cdot \lambda_l \quad \text{for } n > 0 \quad (5.5)$$

$$\alpha_{nh} = P_{nh} \cdot B_n \cdot \lambda_f \quad \text{for } n > 0 \quad (5.6)$$

$$\alpha_{nh} = P_{nh} \cdot B_n \cdot \lambda_p \quad \text{for } n > 0, \quad (5.7)$$

in which λ_g was the probability per unit time of being splashed from the ground at height $n=0$, etc. As each α_{nh} actually consisted of four separate values, four sets of differential equations were included simultaneously in the rain procedure.

Lesion displacement due to growth and rain. In the model, when rain stopped the distribution of conidia at all heights was known. A proportion (X_{sl}) of these conidia produced latent infections. However, a conidium only had the potential to produce a new infection if it was splashed to a site where no infection was already present. Therefore, the probability that each conidium produced a latent infection was estimated by multiplying X_{sl} by a factor representing the probability that a conidium splashed to an unoccupied site.

5.3 The simulations

Vertical spread of light leaf spot in winter oilseed rape was simulated for typical wet and dry seasons. (Meteorological data sets for dry and wet seasons at the Institute of Arable Crop Research, Harpenden, UK from the electronic IACR-Rothamsted archive were used.) Table 5.1 shows the days on which rain events occurred and the duration of each rain event for these dry and wet seasons at the Institute of Arable Crop Research, Harpenden, UK. In addition to weather factors, crop density may influence disease spread. A dense crop has a large PAI, which can decrease the number of conidia splashed to other plant parts so that there is less disease at the end of the stem extension period. To test this, winter oilseed rape crops with low and high densities were simulated separately by either dividing or multiplying the PAI by 2.

Leaf surface area distribution with height may play an important role in the spatial dispersion of conidia during splash events, since it may influence the rate with which conidia can be splashed up to sites at the top of the canopy. To compare light leaf spot spread in winter oilseed rape crops with different leaf surface area distributions, three virtual crops were simulated: one crop had a LAI constant with height, one crop had a LAI increasing linearly with height and one crop had a LAI decreasing linearly with height. In these virtual crops, flowers and pods had the same surface area distribution

Table 5.1: Frequency and duration of rain events on winter oilseed rape during the period of stem extension, for a dry and a wet season. (Data are for the driest and wettest seasons in a period of 30 years at Rothamsted obtained from the Electronic IACR-Rothamsted archive)

Dry season		Wet season			
Day	Rain (min)	Day	Rain (min)	Day	Rain (min)
8	192	1	594	33	30
9	96	2	270	35	606
10	162	3	228	36	48
17	18	5	240	43	396
38	84	8	384	44	246
41	18	9	252	45	528
44	18	10	126	46	246
45	36	11	240	47	54
47	156	12	426	48	36
48	168	13	678	53	444
57	30	14	306	54	342
59	102	16	54	55	300
62	90	18	150	57	288
63	252	19	528	58	48
69	18	20	534	59	414
70	42	24	516	64	54
72	48	25	96	65	306
73	558	26	300	66	270
74	390	28	174	68	396
76	138	29	486	72	78
77	66	30	624	73	138
78	6	31	156	74	192
80	6	32	258	80	96

as the original model winter oilseed rape crop throughout the period of stem extension. Whereas the occurrence and surface area of leaves changed for the original model winter oilseed rape crop, leaf surface area distribution of the three virtual crops was allowed to remain constant during the period. With these simulations, the contribution of splash to disease spread in crops with different LAI was studied. A schematic PAI distribution for the different crops is shown in Figure 5.4. To investigate the actual contribution of upward splash compared to upward disease spread due to stem extension in the winter oilseed rape crop, additional situations were simulated in which either the contribution of upward splash or the contribution of stem extension was omitted. Disease development with time was simulated using combinations of the different conditions. Parameter values were kept constant for each simulation (Table 5.2).

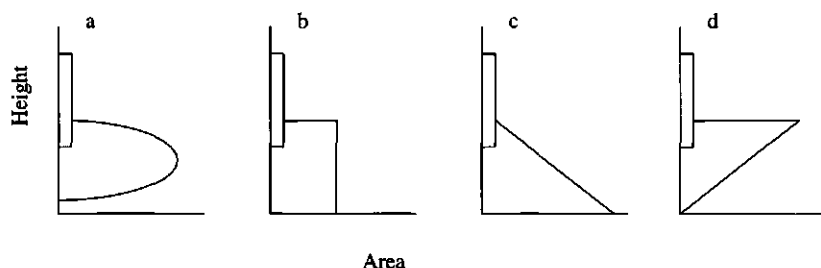


Figure 5.4: Vertical plant surface area distribution for the model winter oilseed rape crop (a) for which surface area distribution of leaves (oval line), flowers and pods (rectangle) changes during the season, and for the virtual crops b, c, and d, for which surface area distribution of the leaves is constant during the season and flowers and pods develop as in (a).

5.4 Results and interpretation

5.4.1 Disease spread in dry and wet seasons

The total numbers of infectious units in the crop during the period of stem extension in a dry and in a wet season generally changed in parallel with plant surface area development (Figure 5.2), i.e. the number of infectious units increased when new plant parts developed and the total number of infectious units decreased rapidly from day 60 onwards when the plant lost many leaves. Lesion numbers increased faster in the wet season than in the dry season. In the dry season fewer conidia were produced and the conidia had less opportunity to move to new sites during rain events. Disease progress depended on young tissue becoming infected before old leaves fell to the ground and lesions were removed from the system, especially during the early period of stem extension (up till day 40). Since conidia were dispersed more frequently in the wet season, the probability that a conidium reached new plant tissue was greater than in the dry season. Thus the difference in epidemic development between situations with disease initiation on the lower or the upper leaves was less in the wet season than in the dry season (Figure 5.5).

When similar simulations were done for crops with different PAI distributions, the difference in disease progress was most apparent when plant density was doubled (Figure 5.5c). The contribution of disease initiated on the lower leaves to the total number of lesions was less for a crop with doubled PAI. When the PAI was less, conidia were more easily splashed to the upper plant parts to infect new tissue. However, disease already present in the upper leaves had more surface area available for spread in the crop with the large PAI than for the crop with small PAI. Sporulating infections in the upper leaves therefore made the largest contribution to disease progress when plant

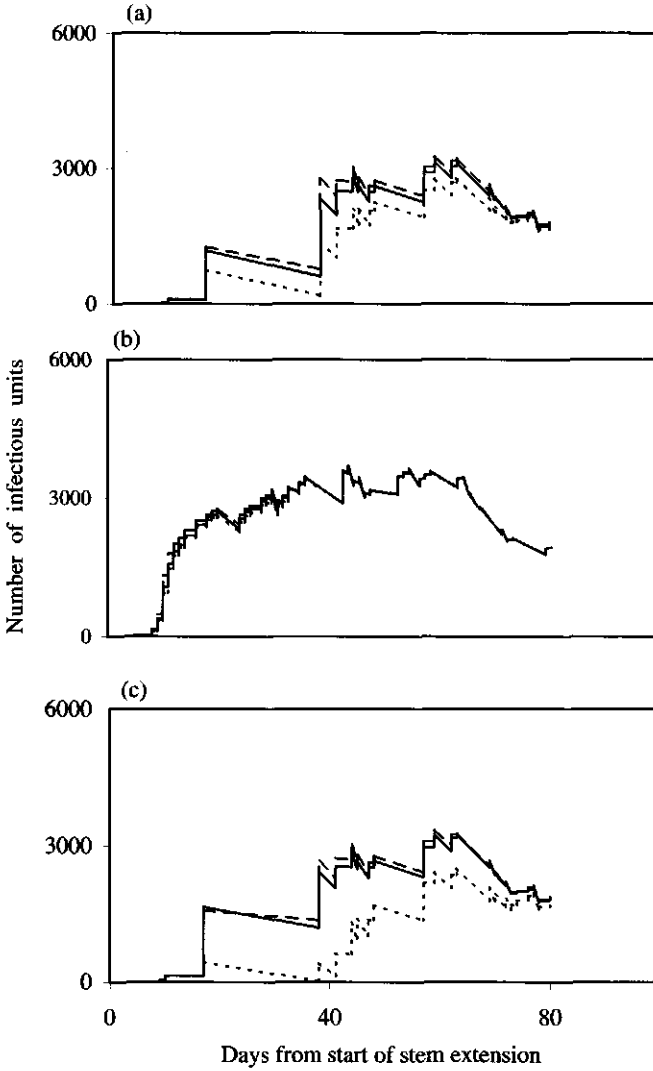


Figure 5.5: Estimates produced by the model for light leaf spot progress (changes in numbers of infectious units with time) in a winter oilseed rape crop in a dry (a) or a wet (b) season and with doubled plant density in a dry season (c) for disease initiated on the lower (dotted lines) or upper (dashed lines) leaves or for disease uniformly distributed (solid lines) at the start of stem extension.

Table 5.2: Default parameter values incorporated in the model.

The probability that a spore will be splashed from:
Ground: 0.25, Leaf: 0.25, Flower: 0.25, Pod: 0.25

And probability that spores will be splashed to this height for different targets.
Splash height in cm:
Ground: 0 (0.5), 10.0 (0.5)
Leaf: 2.5 (0.25), 5.0 (0.5), 15.0 (0.25)
Flower: 0 (1)
Pod: 2.5 (0.5), 5.0 (0.5)

Fraction of:
Sporulating infections that die per day, X_s : 0
Latent infections that become sporulating per day, X_l : 0.059
Spores that become latent infections after the rain event, X_{sl} : 0.003

Number of spores produced by a sporulating infection, N_s : 10^4
--

Surface area of a single infection (cm^2), L_{opp} : 0.36
--

density was increased. In the wet season with doubled plant density, the disease development was very similar to that in the standard crop. In the wet season, rain events were more frequent and therefore more conidia were produced; these conidia had more opportunity to reach the upper leaves in both crops, because frequency and duration of rain increased (data not shown). When plant density was halved, disease incidence increased at a slower rate, particularly until day 40 of the stem extension period, during both the dry and wet seasons (data not shown).

When increase in total number of infectious units in the dry season was simulated for leaves, flowers and pods (Figure 5.6), all available leaf surface was occupied with lesions by about day 60 and the total number of infectious units decreased after this when leaves abscised. The total number of lesions on flowers decreased around day 40, when many flowers abscised and pods developed. At about day 55, all flowers abscised and light leaf spot severity on pods increased rapidly. When flowers first developed (day 20), the proportion of the infectious units on leaves near the top of the canopy was greater when disease was initiated on the upper leaves than when disease was initiated on the lower leaves. This resulted in more infectious units on flowers when disease initiation was on upper compared to lower leaves. At the time of pod development, disease was spread

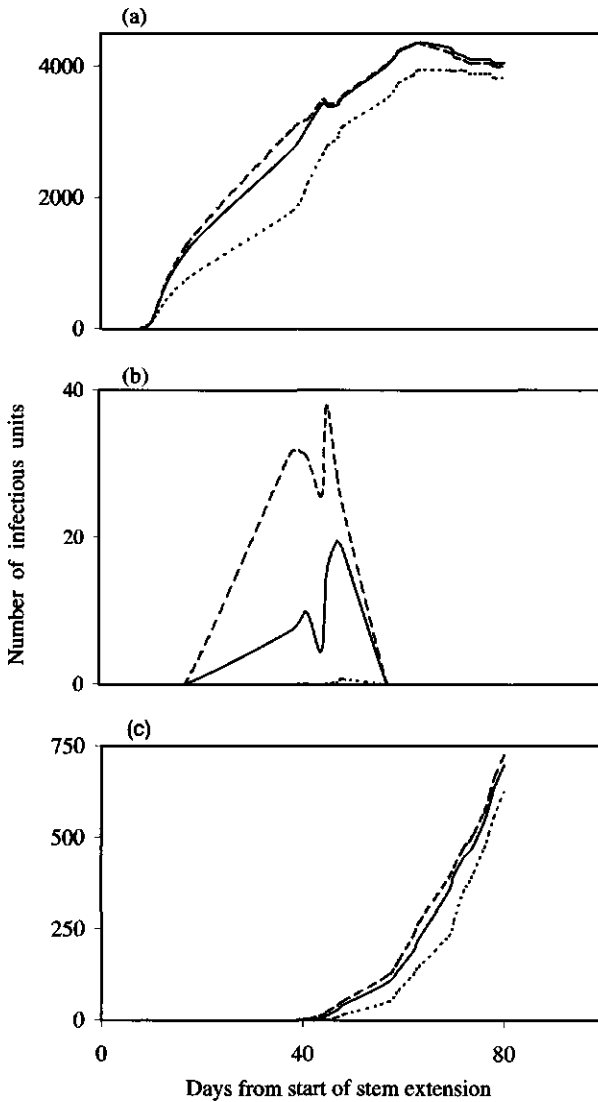


Figure 5.6: Estimates produced by the model for light leaf spot progress (changes in numbers of infectious units with time) separated for leaves (a), flowers (b) and pods (c) in a winter oilseed rape crop in a dry season. Disease was initiated on the lower (dotted lines) or upper (dashed lines) leaves or uniformly distributed (solid lines) at the start of stem extension.

more uniformly over the crop and rain events caused a similar disease development on pods independently of the site of disease initiation at the start of the period of stem extension.

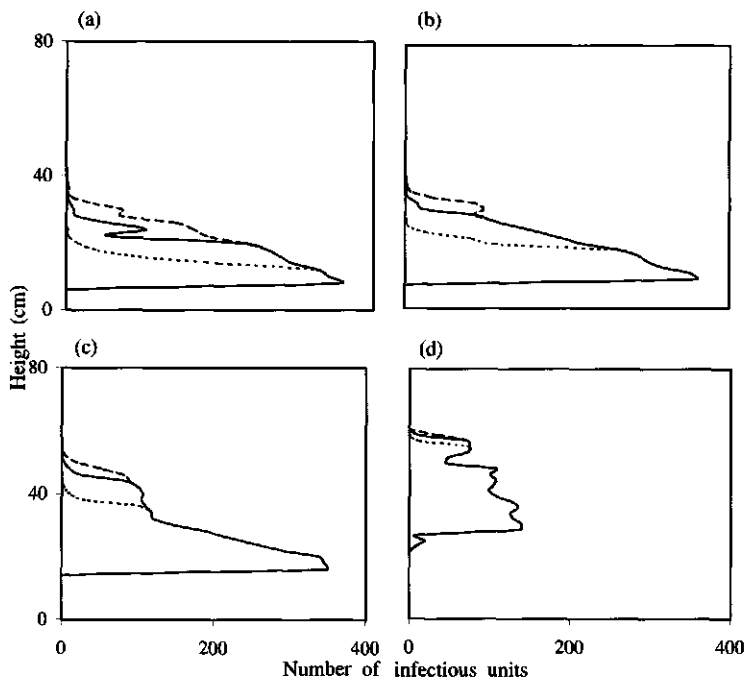


Figure 5.7: Estimates produced by the model of upward light leaf spot spread (changes in numbers of infectious units with height and time) in a winter oilseed rape crop during a dry season for disease initiated on the lower (dotted lines) or upper leaves (dashed lines) and for disease initiated uniformly on the plant (solid lines), illustrated by predicted vertical disease development at 38 (a), 44 (b), 57 (c) or 80 (d) days after the start of stem extension.

The changes in distribution of light leaf spot with height in a winter oilseed rape crop during the dry season were related to the pattern of the surface area development (Figure 5.7). Towards the end of the stem extension period, disease was spread almost uniformly throughout the crop independently of disease initiation site. The same simulation in the wet season resulted in the production of more infectious units which were spread to sites higher in the canopy than in the dry season. The difference in disease development between situations when disease was initiated in the lower or the upper canopy was less in the wet season than the dry season (data not shown). The same trend was observed when plant density was doubled or halved (data not shown).

5.4.2 Effects of leaf surface area distribution

There was a clear difference in disease development between the standard model winter oilseed rape crop and the virtual crops with different LAI distributions (Figure 5.8). The main cause of this difference was that no leaves abscised and no new leaves developed in the virtual crops during the season. The LAI of the standard winter oilseed rape crop decreased during the season because leaves abscised, and lesions were removed from the system so that there were fewer infectious units at the end of stem extension than in the virtual crops. In addition, because a winter oilseed rape crop has a relatively low LAI, conidia can be splashed to sites higher up in the canopy from a site near the ground.

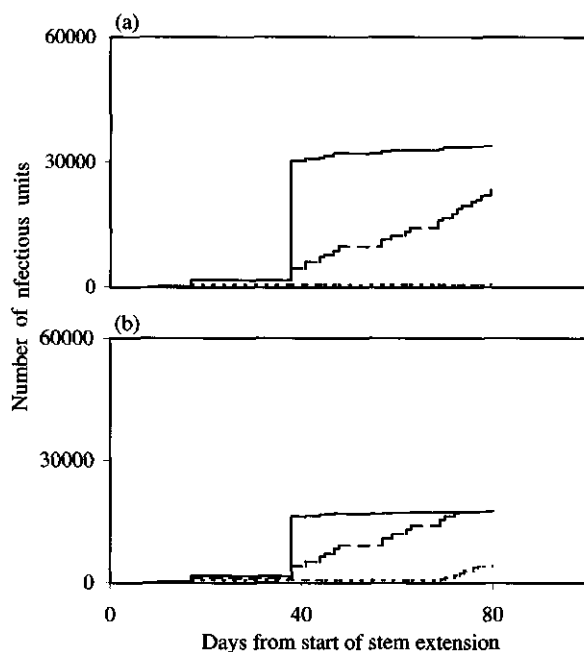


Figure 5.8: Estimates produced by the model of light leaf spot progress in a winter crop having a LAI constant with height (a) and in a crop having a LAI decreasing with height (b) in a dry season. Disease was initiated on the lower leaves (dotted lines), upper leaves (dashed lines) uniformly on the plant (solid lines) at the start of stem extension.

In the virtual crops with a constant LAI (Figure 5.8a) or decreasing LAI with height (Figure 5.8b), in a dry season the large LAI near the ground prevented conidia from being

more uniformly over the crop and rain events caused a similar disease development on pods independently of the site of disease initiation at the start of the period of stem extension.

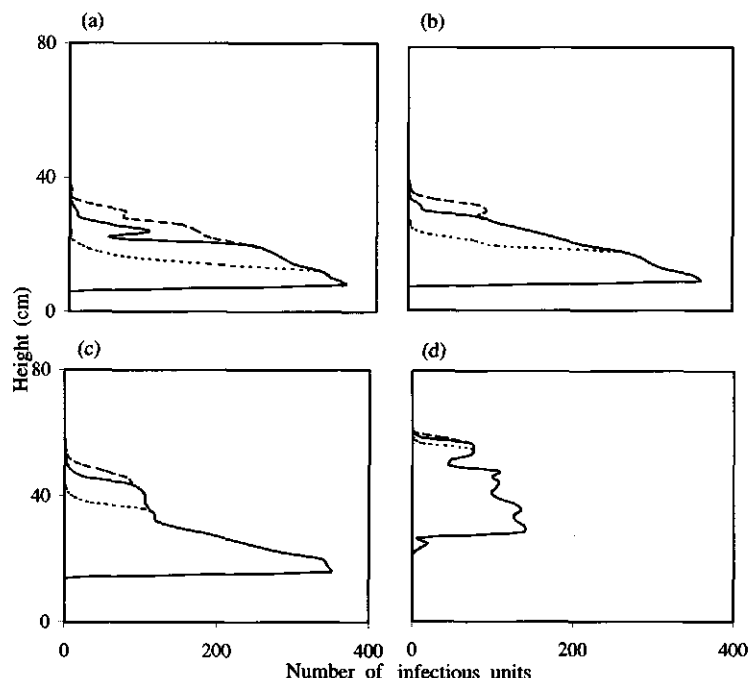


Figure 5.7: Estimates produced by the model of upward light leaf spot spread (changes in numbers of infectious units with height and time) in a winter oilseed rape crop during a dry season for disease initiated on the lower (dotted lines) or upper leaves (dashed lines) and for disease initiated uniformly on the plant (solid lines), illustrated by predicted vertical disease development at 38 (a), 44 (b), 57 (c) or 80 (d) days after the start of stem extension.

The changes in distribution of light leaf spot with height in a winter oilseed rape crop during the dry season were related to the pattern of the surface area development (Figure 5.7). Towards the end of the stem extension period, disease was spread almost uniformly throughout the crop independently of disease initiation site. The same simulation in the wet season resulted in the production of more infectious units which were spread to sites higher in the canopy than in the dry season. The difference in disease development between situations when disease was initiated in the lower or the upper canopy was less in the wet season than the dry season (data not shown). The same trend was observed when plant density was doubled or halved (data not shown).

5.4.2 Effects of leaf surface area distribution

There was a clear difference in disease development between the standard model winter oilseed rape crop and the virtual crops with different LAI distributions (Figure 5.8). The main cause of this difference was that no leaves abscised and no new leaves developed in the virtual crops during the season. The LAI of the standard winter oilseed rape crop decreased during the season because leaves abscised, and lesions were removed from the system so that there were fewer infectious units at the end of stem extension than in the virtual crops. In addition, because a winter oilseed rape crop has a relatively low LAI, conidia can be splashed to sites higher up in the canopy from a site near the ground.

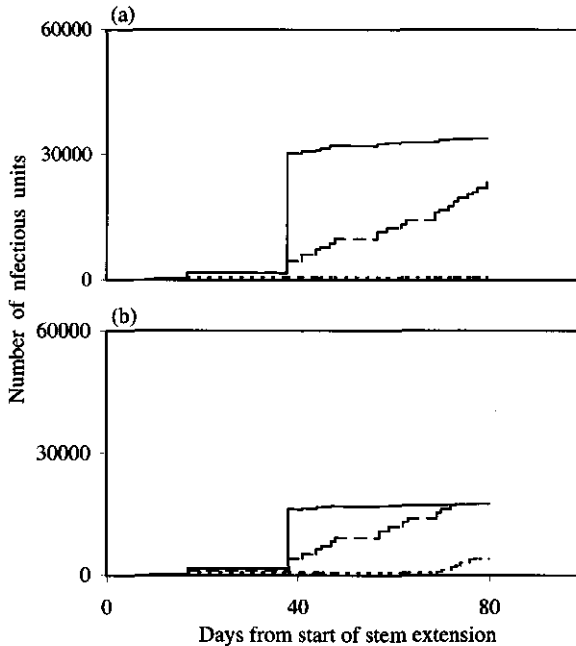


Figure 5.8: Estimates produced by the model of light leaf spot progress in a winter crop having a LAI constant with height (a) and in a crop having a LAI decreasing with height (b) in a dry season. Disease was initiated on the lower leaves (dotted lines), upper leaves (dashed lines) uniformly on the plant (solid lines) at the start of stem extension.

In the virtual crops with a constant LAI (Figure 5.8a) or decreasing LAI with height (Figure 5.8b), in a dry season the large LAI near the ground prevented conidia from being splash dispersed to the upper canopy and disease progress was limited. Conidia reached

other plant parts more easily when disease was initiated on the upper leaves. A uniform distribution of lesions at the start of stem extension produced disease development at all available sites in the canopy. A crop having a LAI constant with height was then most favorable for disease development. The surface area was large enough at all heights to provide sites on which new lesions could be produced and conidia could be splashed to other plant parts more easily than in other virtual crops. The maximum number of infectious units was therefore soon reached on all plant parts.

The simulations suggested that disease present on the lower leaves could not easily spread up crops when LAI increased with height. A uniform disease distribution or disease on the upper leaves at the start of stem extension in the crop produced similar results to those for the crop with a LAI decreasing with height (data not shown). Although the total number of lesions increased faster, similar results were observed for the virtual crops during wet seasons (data not shown).

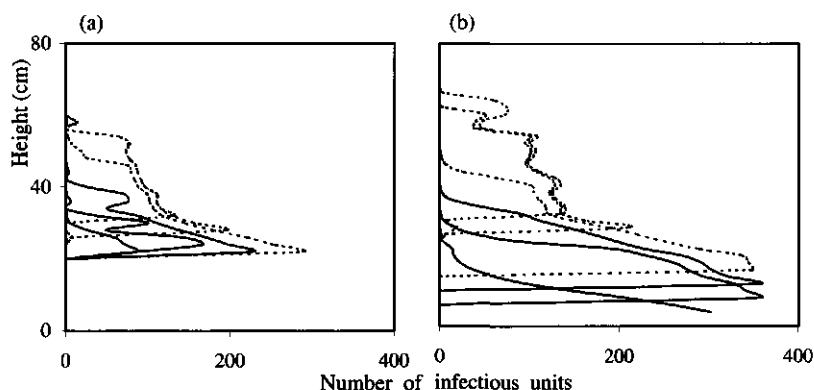


Figure 5.9: Estimates produced by the model of upward light leaf spot spread in a winter oilseed rape crop when dispersal was simulated only due to stem extension (a) or rain-splash (b). Disease was initiated on the upper leaves (a) or uniformly distributed (b) at the start of stem extension. (Separate lines indicate numbers of infectious units, with the disease curve presented from a solid line to dotted towards the end of the stem extension period).

5.4.3 The interaction between crop growth and rain events

The relative importance of stem extension compared to splash dispersal for upward spread of light leaf spot was studied by simulating a period of winter oilseed rape growth in which rain could produce a fixed number of conidia splashing within the same layer. Conidia could be dispersed by splash to the apex where new plant parts developed, but

not to other heights in the canopy. The contribution of extension growth to disease dispersal with conidia only able to splash within the same layer is presented in Figure 5.9 a. The contribution of splash to disease development without vertical disease displacement due to extension growth is shown in Figure 5.9 b. As conidia had a fixed probability of being splashed from a leaf, flower or pod to one of these parts at a different height in the canopy (Table 5.2), the disease spread throughout the canopy when splash was the only mechanism of upward disease development. However, disease spread was slower and less disease was spread per unit time when the displacement of lesions due to stem extension was omitted. In these simulations there was a clear interaction between the two mechanisms of upward disease spread in a winter oilseed rape crop throughout the season. Rain events contributed to the production of conidia which were splashed to the plant apex and moved vertically by internode growth.

5.5 Discussion

This study showed the importance of rain-splash in the upward spread of light leaf spot on winter oilseed rape during the period of stem extension. The simulations of light leaf spot development under different conditions suggested that there are several interactions between the two mechanisms (rain-splash and stem extension) during this period of upward disease spread, i.e.

Horizontal splash of conidia to the plant apex and subsequent upward spread by stem extension is a major mechanism for the dispersal of light leaf spot in a winter oilseed rape crop. Rain events contributed to the production of infectious units, which were either directly splashed to the upper canopy, or to the plant apex and moved to new developing tissue by internode growth. Frequency and duration of rain events influenced the infection process, with fewer conidia being produced and less opportunity to move to new sites in a dry season. This interaction affected other disease progress results, i.e:

Total numbers of infectious units changes in parallel with plant surface area development. New leaves developed during the first two months of the stem extension period. Rainfall during this period played a crucial role in disease progress. Leaf abscission before a next rain event reduced the movement of infectious units to the upper canopy and if the period without rain would be long enough in a period of many leaf abscissions, the crop would have the potential to even outgrow major disease progress to the pods.

Light leaf spot development on the upper leaves plays a major role in total disease progress when crop density increases. Disease spread to the upper leaves depended not only on the frequency and duration of rain events, but also on the LAI. An increased surface area prevented conidia of being transported to new developing plant tissue. However, infectious units that did reach the upper leaves had more surface area available for disease progress, which resulted in a faster disease progress than in the original winter oilseed rape crop. The effect of crop density particularly played an important role at the

beginning of stem extension when leaf abscission before a next rain event together with a doubled LAI prevented a rapid disease progress of infectious units on the lower leaves.

Light leaf spot progression to the pods depends on disease distribution on the leaves. The number of infectious units that reached the upper leaves by the time of pod development rather than the number of infectious units on the flowers determined disease severity on pods. Simulation results showed a clear difference in disease severity on the flowers, bearing more infectious units when relatively more disease was present on the upper compared to the lower leaves at the time of flower development. This difference was not visible on the pods which developed from the flowers, indicating that a majority of the infectious units on the pods were splashed from the leaves which had a more uniform distribution of infectious units at the time of pod development. Su et al. (1998) reported that models relating yield to incidence of light leaf spot on leaves at growth stage 3.3 accounted for more of the variance than models at earlier or later growth stages.

Simulation results were compared with light leaf spot development observations on winter oilseed rape crops at the Institute of Arable Crop Research, Harpenden, Uk (Rothamsted 1996-1999, unpublished), with disease progress on potted plants outside at the Institute of Arable Crop Research, Harpenden, Uk (Rothamsted 1998/ 1999, unpublished) and with data in the literature (Jeffery et al. 1989; Su et al. 1998 and Stinchcombe et al. 1986), Table 5.3. The model predicted a fast disease progress in wet periods and a more gradual development when the period of stem extension was relatively dry. Most of the disease was however on the lower leaves and the maximum number of infectious units on the upper 4 to 5 leaves was only reached in May in a wet period.

With respect to lesion development, Figueroa et al. (1995) found lesion numbers in the range of 6.8 per 20cm² leaves for a resistant winter oilseed rape cultivar up to 28 per 10cm² leaves for a more susceptible cultivar. This would correspond to a maximum in the range 493 - 4060 lesions in the model plant, having a maximum total surface area of c. 1450cm² (Figure 5.2). Simulation results lie within that range (Figure 5.5).

Additional field assessments and experimental research is needed to establish the relative importance of factors potentially influencing the development of light leaf spot on winter oilseed rape. Once the impact of these factors on disease spread is known the model can be extended for those processes which have a major effect on disease development throughout the growing season. In the next stage the relative contribution of included mechanisms can be assessed by varying the input variables (Table 5.2). Ultimately, simulation results can provide important information in developing disease management strategies. However, ultimately for practical warning purposes the model should be validated with disease progress data from the field. The model input should then consist of initial disease conditions as recorded in the field at a given time in early spring and disease development would subsequently be followed throughout the season as a consequence of the rain events that occurred in the season.

Table 5.3: Progress of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape, expressed as the percentage areas of total plant, lower leaves, upper 4 to 5 leaves or pods of the terminal covered with sporulating *P. brassicae*. Data show model outputs, observations at Rothamsted (1996-1999; 1998-1999, unpublished) and published assessments.

	Model outputs		Rothamsted data		Jeffery et al.'89 range	Su et al. '98 range	Stinchcombe et al. '86
	dry	wet	'96-'99 range	'98-'99 range			
<i>Total:</i>							
March ^a	0.1	0.1	0.5 - 45			3 - 30	
April	46	95				16 - 63	
May ^b	93	100	0.1 - 50			12 - 32	
June ^c	100	100	0 - 90			7 - 9	
<i>Lower leaves:</i>							
March ^a	0.1	0.1		30 - 90			
March ^d	0.45	71		20 - 80			
May ^b	83.8	100					
<i>Upper leaves:</i>							
March ^a	0.1	0.1		0 - 0			
March ^d	0.05	2.23		0 - 0	4.5 - 11		
April	0	9.2			11 - 18		2.5
May ^b	9.2	100			13 - 23		10.7
<i>Pods:</i>							
May ^e	0.89	65	0 - 50	0 - 0.5			
June ^c	60	91	0 - 80				31

^aAt the start of stem extension

^bBefore mid-May

^cAt the end of stem extension

^dAt the end of March

^eAfter mid-May

Acknowledgements

We thank the Life Sciences Foundation (SLW project 05-39-181), which is supported by the Netherlands Organization for Scientific Research (NWO), the UK Biotechnology and Biological Sciences Research Council and the UK Ministry of Agriculture, Fisheries and Food and Home-Grown Cereals Authority for funding. We thank G.W. Heusinkveld for assisting with programming, T. Gilles for giving information on light leaf spot development in a winter oilseed rape crop and J. Steed for providing experimental data.

Chapter 6

A theory on the vertical dispersal of splash-borne pathogen units influenced by arable crop characteristics

A. Pielaat F. van den Bosch M. de Gee N.J. van der Wal

Abstract

An analytical mechanistic model was proposed to study the vertical spread of splash-borne spores in arable crop canopies. Three crop types were considered, with different LAI distributions. The influences of crop characteristics and rain properties on vertical spread were investigated. The LAI affected the amount of rain being intercepted by the canopy and the vertical displacement of splashed spores. Splash dispersal was concentrated in the upper canopy layers in a crop having LAI constant or increasing with height. Splash probabilities were greatest and most spores were intercepted in the layers just beneath the upper layers in a crop having LAI decreasing with height.

6.1 Introduction

Spores of many plant pathogens are dispersed in the splash droplets which are formed when raindrops hit the water film formed on the surface of arable crop canopies during rain events. Important fungal plant pathogens for which the main dispersal mechanism

is by rain-splash are *Phytophthora* spp. on citrus, rhododendron, apples, black pepper and strawberry, *Colletotrichum* spp. on citrus, onion, rice and strawberry, *Fusarium* spp. on cereals, *Pseudocercospora* spp. on wheat and oilseed rape, *Septoria* spp. on wheat and tomatoes and *Diaporthe* spp. on soybean. However, the spatial spread of spores in a canopy by rain-splash is affected not only by fungus-specific mechanisms for spore release and the properties of the rain. Different plants each have characteristic morphologies, which play an important role in spore dispersal, because of the different wetting of surface areas in the canopy. Potential splash events depend on the PAI (Plant Area Index, total surface area of plant tissue per surface area of ground; Pielaat, van den Bosch, Fitt and Jeger, chapter 5).

In crops, infected plant debris on the soil surface often forms the primary source of inoculum for these pathogens and gradual upward and lateral spread of disease in the canopy is by rain-splash (Ramachandran *et al.*, 1990 and Linders *et al.*, 1996). The influence of soil types on disease spread has formerly been investigated for a variety of soils and crops (Berrie and Luton, 1996; Madden and Ellis, 1990; Yang and TeBeest, 1992). Further spread in the canopy has also been studied for different crops and different results have been reported (Okayama, 1994; Ferrandino and Elmer, 1996; Fitt *et al.*, 1992 and Freitas *et al.*, 1998).

The objective of those experiments has been to understand the spatial spread of disease in different plant-pathogen systems, so as to improve disease control procedures. For example both chemical and biological treatments have been introduced to control diseases spread by rain-splash (Stobart *et al.*, 1999; Chauhan and Singh, 1991; Soleimani *et al.*, 1996). Another option has been the introduction of physical barriers, which was tested by Okayama (1994) in strawberry crops. Nevertheless, optimal disease management strategies can be developed only when mechanisms of disease spread in all of these crops during the growing season have been quantified. Disease spread in the different crops, with a wide range of specific morphological characteristics which change during the growing season, cannot be quantified adequately by experimental research alone. The development of physical models helps to improve understanding of the general mechanisms of splash dispersal of pathogens in a range of crops. A simulation model has been developed to study the vertical spread of light leaf spot on winter oilseed rape, where both plant growth and rain-splash influenced disease dispersal (Pielaat, van den Bosch, Fitt and Jeger, chapter 5). It was shown that spores were splashed to the plant apex and directed upwards by stem extension.

In this paper an analytical model is proposed and the influence of crop canopy structure on the vertical dispersal of splash-borne pathogens is investigated, for crops with Leaf Area Index (LAI, cm^2 leaf per cm^2 ground per cm plant height) constant, increasing or decreasing with height above the ground. For this purpose the influence of rain properties on the separate terms in this model describing the physical splash process are investigated qualitatively for the three crops.

6.2 The model

Consider a crop in which spores are dispersed vertically in the canopy by rain-splash. Spores are splashed to sites on the upper and lower leaves or to the ground, and re-splashed on the plants during rain events. This process was modeled by subdividing the splash dispersal process into its component mechanisms, 1. A raindrop hits the water film formed on a leaf or the ground and spores are dispersed in the splashing rain droplets. 2. Splashed spores are redistributed in the crop and on the soil surface by secondary splash. These two mechanisms were translated into probabilities and formed the basis of the model. A distinction was made between the probabilities that a rain droplet was splashed from the ground and splashed from the plant leaves in the crop because the physical surface structure of plants differed from that of the ground. In addition, spores splashing from the ground can only be dispersed upwards into the canopy or horizontally to stay on the ground, whereas spores splashing from plants can be dispersed both upwards and downwards in the canopy. Two separate equations were therefore formulated for redistribution of spores splashing from the ground and spores splashing from the plants.

First let us consider the redistribution of spores present at a certain height h (for $h > 0$) in the canopy during a rain event. The spore density at height h and time t during a rain event was denoted by $H_{h,t}$ (i.e. number of spores per LAI per cm plant height at height h). By multiplying $H_{h,t}$, with the probability per unit time of a spore being hit (γt^{-1}) and a splash probability (λ_h), the density of spores that splash away from height h per unit time (first term on the right in eq. 6.1) was described.

In addition, a fraction of the spores splashed from other heights in the crop during this time are deposited at height h . The water surface surrounding the ground is hit and subsequently spores are splashed from the ground with a probability $\gamma\lambda_g t^{-1}$ and from any other height in the crop with a probability $\gamma\lambda_n t^{-1}$. A probability density function (p.d.f.) $D_{n,h}$ described the likelihood that a spore was deposited at height h when splashed from any height n . Multiplying $\gamma\lambda_g t^{-1}$ by $D_{0,h}$ gave the fraction of spores being deposited at height h when splashed from the ground per unit time during the rain event. The integral of $D_{n,h} \gamma\lambda_n t^{-1}$, with respect to n , gave the fraction of spores deposited at height h when splashed from any other site n in the crop (where $n > 0$). The actual number of spores deposited at height h in the splash process was obtained by multiplying the appropriate dispersal function by the spore density on the ground (i.e. number of spores per cm^2 soil at time t) denoted by G_t (last term on the right in eq. 6.1), and by spore density at any other height in the crop ($H_{n,t}$) respectively (second term on the right in eq. 6.1). The vertical redistribution of spores on the plants per unit time during a rain event was therefore formulated as

$$\frac{\partial H_{h,t}}{\partial t} = -\gamma_t \lambda_h H_{h,t} + \int_0^{\max} \gamma_t \lambda_n H_{n,t} D_{n,h} dn + \gamma_t \lambda_g G_t D_{0,h}, \quad (6.1)$$

where \max represents the maximum height of the crop (in cm).

By analogy with spore redistribution on the plants, a mathematical expression was derived for the change in the number of spores on the soil surface. This redistribution was defined by the number of spores that splashed away from the ground and the number of spores that splashed from the plants or the ground to the ground per unit time (\mathcal{X}). Thus

$$\frac{dG_t}{dt} = -\gamma_t \lambda_g G_t + \mathcal{X}. \quad (6.2)$$

An expression for \mathcal{X} was found when the total number of spores during a rain event was considered. If no spores are lost from the system, the total number of spores in the crop and on the ground is constant in time, so

$$\frac{dG_t}{dt} + \int_0^{\max} \frac{\partial H_{h,t}}{\partial t} dh = 0. \quad (6.3)$$

Substituting equations 6.1 and 6.2 in equation 6.3 gave

$$\begin{aligned} -\gamma_t \lambda_g G_t + \mathcal{X} - \int_0^{\max} \gamma_t \lambda_h H_{h,t} dh + \int_0^{\max} \gamma_t \lambda_n H_{n,t} \int_0^{\max} D_{n,h} dh dn \\ + \gamma_t \lambda_g G_t \int_0^{\max} D_{0,h} dh = 0. \end{aligned} \quad (6.4)$$

$$\text{As } \int_0^{\max} D_{0,h} dh = 1 - D_{0,0},$$

$$X = \int_0^{\max} \gamma_t \lambda_h H_{h,t} dh - \int_0^{\max} \gamma_t \lambda_n H_{n,t} \int_0^{\max} D_{n,h} dh dn + \gamma_t \lambda_g G_t D_{0,0}. \quad (6.5)$$

This indicates that the number of spores reaching the ground per unit time consists of the spores that are not intercepted by the crop when splashing from the plants, and spores that are redeposited on the ground when splashing from the ground.

With this model (6.1) and (6.2), the influence of crop characteristics on the vertical dispersal of pathogen spores during rain events was studied. Crop types differed in leaf structure and leaf surface area distribution and this affected the splash process. Therefore, both the probabilities that a spore was splashed and that a spore was deposited

differed between crops. Crop characteristics were expressed by the parameters λ_h , λ_g and the p.d.f. $D_{n,h}$ for spore deposition. In this research three crop types, which differ in leaf area distribution, were considered. The three crops had a Leaf Area Index (LAI, cm^2 leaf per cm^2 ground per cm crop height) constant, decreasing or increasing with height. Expressions for λ_h , λ_g and $D_{n,h}$ were derived from a simple stochastic model for the different crop types in order to study the splash process.

6.3 Results and Discussion

6.3.1 The probability that a spore was splashed in different crops.

Before a spore can be splash dispersed from a particular layer with thickness ξ in the crop, a raindrop has to penetrate the canopy down to this layer. It was assumed that the likelihood that a raindrop penetrated the crop down to the base of the canopy was proportional to the LAI the raindrop encounters from the point where it entered the crop. Thus,

$$\frac{dP_h}{dh} = LAI_h P_h, \quad P_{max} = 1$$

The probability that a raindrop penetrated the crop to below height h was therefore

$$e^{-\int_h^{max} LAI_\sigma d\sigma}$$

A raindrop will be intercepted in the crop canopy layer between h and $h-\xi$ according to $P_h - P_{h-\xi}$ and so the probability that a raindrop was intercepted in this layer became

$$e^{-\int_h^{max} LAI_\sigma d\sigma} (1 - e^{-\int_{h-\xi}^h LAI_\sigma d\sigma}).$$

With the Taylor approximation for $1 - e^{-\int_{h-\xi}^h LAI_\sigma d\sigma}$, it followed that

$$e^{-\int_h^{max} LAI_\sigma d\sigma} LAI_h \xi.$$

The probability that a raindrop was intercepted in the layer between h and $h-\xi$ was multiplied by the probability that a spore was incorporated into the splash droplets (l_h)

to give the probability ($\lambda_h \xi$) that a spore was splashed from this layer with thickness ξ in the canopy, and therefore

$$\lambda_h = l_h LAI_h e^{-\int_h^{max} LAI_\sigma d\sigma} \quad (6.6)$$

In equation (6.6) the term $e^{-\int_h^{max} LAI_\sigma d\sigma}$ can be interpreted as the probability that a raindrop was not intercepted between the point where it entered the canopy (max) and height h . ξLAI_h indicates interception between h and $h-\xi$. The parameter l_h was replaced by l_g when spores were splashed from the ground (where $h=0$).

Crop characteristics, expressed in terms of LAI and the leaf structure, influence the value of λ_h . Different equations for λ_h were therefore derived from the different LAI distributions of the three crops in Appendix A. The equations (6.8), (6.9) and (6.10) of Appendix A were used for the analysis.

Figure 6.1 shows the distributions for probabilities that a spore was splashed from different heights in the canopy (where $h > 0$) when different values of c (the total LAI of a plant) and different values for l_h (the rebound probability) were applied. Raindrops can penetrate the canopy less easily when relatively more surface area is present in the upper layers. Many raindrops were already intercepted in the upper layers of a crop having a constant LAI. The probability that a spore was splashed from a height layer therefore decreased rapidly with decreasing height in this crop (Figure 6.1 a,b), particularly when the total LAI, c , was large (Figure 6.1b). The effect of having more surface area in the upper layers was even stronger in a canopy having an increasing LAI. Therefore, splash probabilities decreased rapidly towards the ground in this canopy (Figure 6.1 e,f). When the crop had a decreasing LAI the probability that a spore was splashed first increased down to some layer and then decreased below this layer (Figure 6.1 c,d). The LAI was large and it was less likely for a drop to penetrate to the lower layers of this crop. A large value of c increased the probability that a spore was splashed from the upper leaves (Figure 6.1 b,d,f) relative to the crops with a low value of c (Figure 6.1 a,c,e). The total probability distribution increased when the probability of a spore being rebounded (l_h) increased.

6.3.2 Vertical spread of splashed spores in different crops.

By analogy with the procedure for obtaining expressions for λ_h in the three crops, equations for the p.d.f. $D_{n,h}$, describing the likelihood that a spore was deposited between height h and $h-\xi$ when splashing from height n with thickness ξ , were formulated.

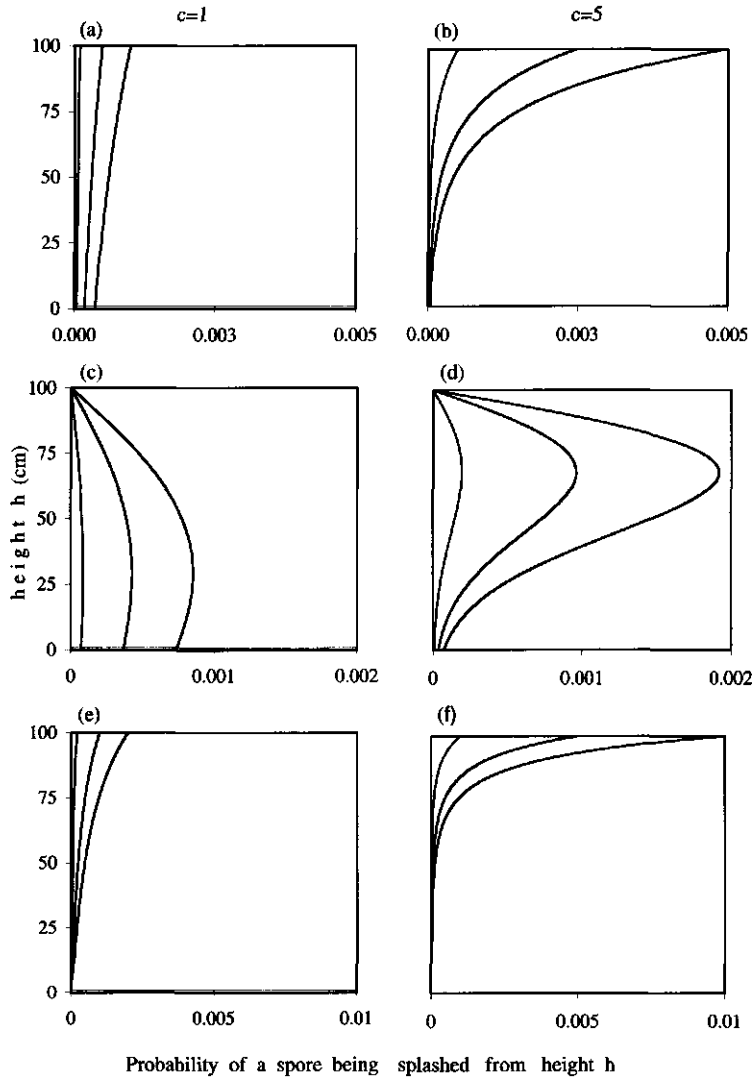


Figure 6.1: The probability of a spore being splashed from height h with thickness ξ in a crop having LAI constant (a,b),decreasing (c,d), or increasing (e,f) with height, and a total LAI, $c=1$ (a, c, e) or 5 (b, d, f) and a rebound probability, $l_h=0.1$ (left line), 0.5 (middle line) or 1.0 (right line). Crop height was 100 cm.

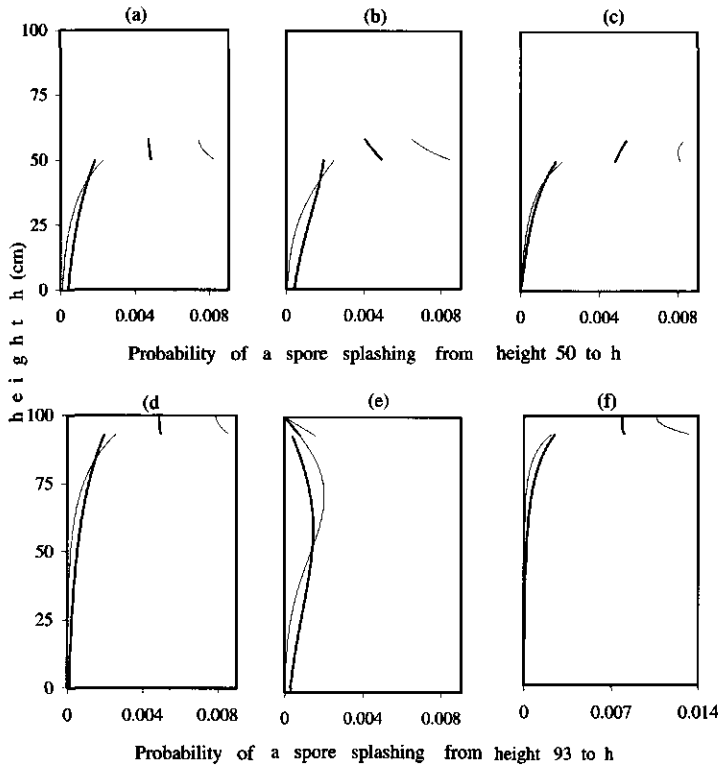


Figure 6.2: P.d.f. for the probability that a spore is deposited at height h with thickness ξ when splashed from height category $n=50$ cm (a,b,c) or $n=93$ cm (d,e,f) in a crop having LAI constant (a,d), decreasing (b,e) or increasing (c,f) with height, and a total LAI, $c=3$ (solid line) or 6 (dotted line). Crop height was 100 cm and splash height was 8 cm.

The term

$$e^{-\int_n^{n+a} LAI_\sigma d\sigma}$$

was used to describe the likelihood that a spore was not intercepted between height n and its maximum splash height $n+a$. The mean maximum height (a) of spore splashes was calculated from experimental results in which ballistic trajectories of spores splashing from different plant parts were estimated (Pielaat, Marshall, McCartney, van den Bosch and Fitt, chapter 4). The p.d.f. $D_{n,h}$ for spore deposition was composed of three parts; a spore can be deposited at a vertical distance which is higher than, lower than or equal to the original point of drop impactation. Therefore

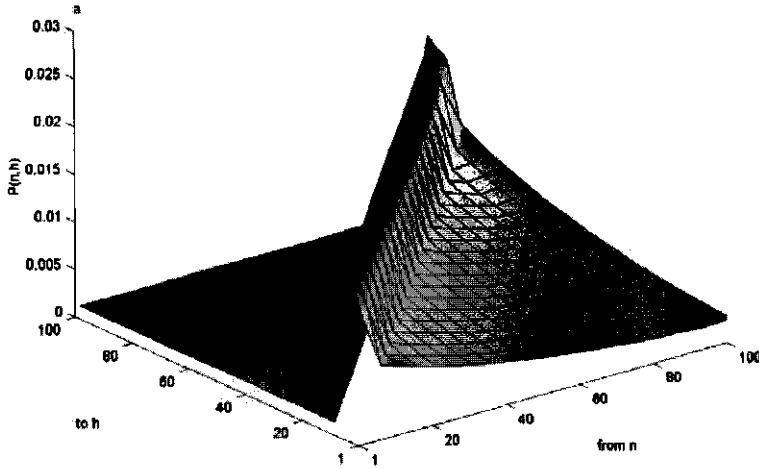


Figure 6.3: Probability density function for a spore being deposited at height h with thickness ξ when splashed from height n with thickness ξ in a crop having a with height constant LAI and a total LAI, $c=2$. Crop height was 100 cm and splash height was 8 cm.

$$D_{n,h} = \begin{cases} \xi LAI_h \left(e^{-\int_n^h LAI_\sigma d\sigma} + e^{-\int_n^{n+a} LAI_\sigma d\sigma} e^{-\int_h^{n+a} LAI_\sigma d\sigma} \right) & \text{if } h > n \\ \xi LAI_h e^{-\int_n^{n+a} LAI_\sigma d\sigma} e^{-\int_h^{n+a} LAI_\sigma d\sigma} & \text{if } h < n \\ \xi LAI_h \left(1 + e^{-2 \int_n^{n+a} LAI_\sigma d\sigma} \right) & \text{if } h = n \end{cases} \quad (6.7)$$

The term $n+a$ was replaced by \max when spores splashed from a height where $\max < n+a$. A more detailed explanation of the p.d.f. $D_{n,h}$ can be found in Pielaat, van den Bosch, Fitt and Jeger, chapter 5. When the corresponding LAI distributions for the three crops were substituted in these equations, different p.d.f.s for spatial spread were obtained as described in Appendix B. The equations of Appendix B were used for the analysis.

Figure 6.2 shows examples of the p.d.f.s for the vertical spread of a spore splashing either from a layer at height 50 cm in the canopy, where $\max > n+a$, or from a layer at height 93 cm in the canopy, where $\max < n+a$. Transition probabilities were plotted for

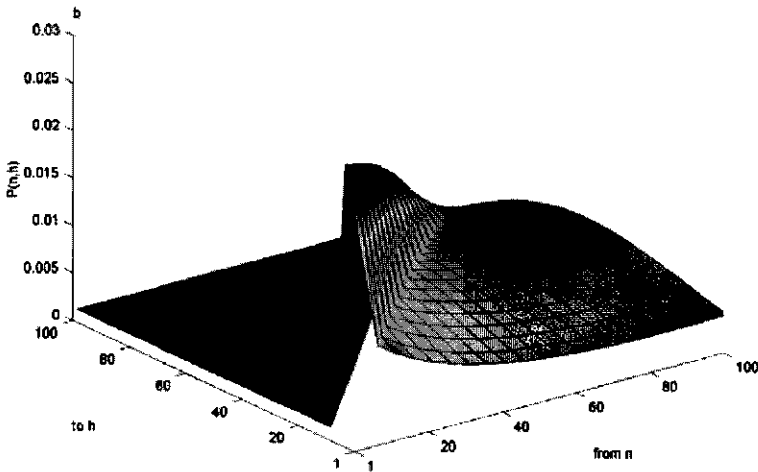


Figure 6.4: Probability density function for a spore being deposited at height h with thickness ξ when splashed from height n with thickness ξ in a crop having a with height decreasing LAI and a total LAI, $c=2$. Crop height was 100 cm and splash height was 8 cm.

the three different crops (where $h > 0$) and two values of c (the total LAI of a plant). A maximum mean splash height of 8 cm was used. The largest deposition probabilities were in the height layers above or level with the original drop impact point, with more spores being deposited when the LAI in the layers above the impact point increased and when the total LAI, c , of a crop increased. Apparently the LAI above each impact point was large enough to incorporate many of the splashed spores. The greater the surface area above the impact point the greater the deposition probabilities. As a consequence the probability of a spore being deposited increased towards the maximum splash height when a spore was splashed from 50 cm in a crop having an increasing LAI (Figure 6.2c). This trend was not observed when spores were released from a layer at height 93 cm in this crop. The LAI was already so large at height 93 cm that transition probabilities had a maximum value just above the impact point when splashed from this layer.

Although individual transition probabilities were smaller, most of the spores released were splashed to a layer beneath the impact point. The LAI below each impact point was large enough to prevent many splash droplets from penetrating down to the ground. Splash to the lower layers decreased gradually with decreasing deposition probabilities towards the ground when the LAI, c , was large (Figure 6.2 a,b,c,d,f). The more surface area just beneath the impact point the faster the deposition probabilities decreased.

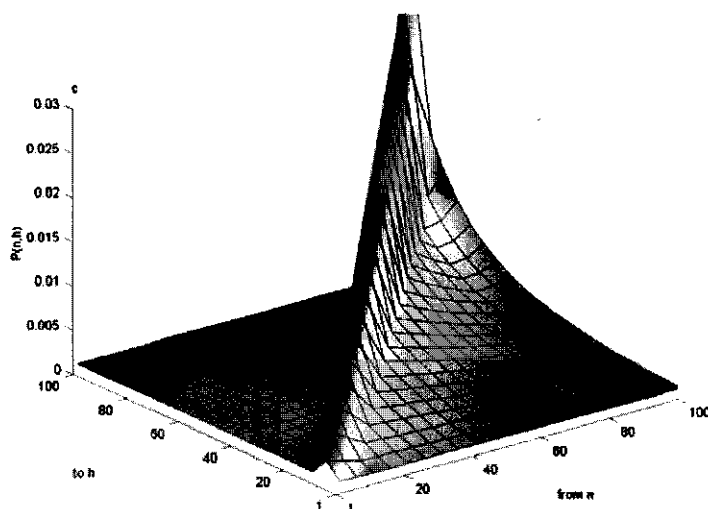


Figure 6.5: Probability density function for a spore being deposited at height h with thickness ξ when splashed from height n with thickness ξ in a crop having a with height increasing LAI and a total LAI, $c=2$. Crop height was 100 cm and splash height was 8 cm.

An exception to this result was observed in a crop having LAI decreasing with height, where splash to the lower layers first increased and then decreased. The surface area was too low in the upper layers for spore deposition in a crop having a decreasing LAI, therefore more spores were deposited in the lower canopy with relatively slow decreasing probabilities when the total LAI was low. This trend was more apparent when the total LAI, c , was larger.

The p.d.f.s for deposition in height layer h (for $h > 0$) when splashing from an arbitrary layer n (for $n > 0$) are shown in Figures 6.3, 6.4 and 6.5 for the three different crop types. Transition probabilities are largest in those layers having the largest LAI.

In this paper we have shown how leaf area index distribution (constant, decreasing, or increasing with height) affect key parameters involved in the splashing of spores within and beneath a crop canopy. This should form a basis for evaluating the consequences of breeding programmes leading to different plant architecture or new cropping practices where splash-dispersed pathogens are constraints to production.

Acknowledgements

The investigations for project 05-39-181 were supported by the Life Sciences Foundation (SLW), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

Appendix A

The LAI distribution was specified for three crop types to study the influence of crop morphology on the probability that a spore was splashed from height h with thickness ξ per time unit during a rain event.

Crop 1

The first crop had LAI constant with height, defined by

$$LAI_h = \begin{cases} c & \text{for } h \leq max \\ 0 & \text{for } h \geq max \end{cases}$$

So, $\int_0^{max} LAI_\sigma d\sigma = c max$ and

$$\lambda_h = l_h \xi c e^{-c(max-h)}. \quad (6.8)$$

Crop 2

The second crop had a LAI decreasing with height, defined by

$$LAI_h = \begin{cases} \alpha(max-h) & \text{for } h \leq max \\ 0 & \text{for } h \geq max \end{cases}$$

So, $\int_0^{max} LAI_\sigma d\sigma = \int_0^{max} \alpha(max-\sigma)d\sigma$. Since the three crops did not differ in total LAI, $\alpha = \frac{2c}{max}$ and

$$\lambda_h = l_h \xi 2c \left(1 - \frac{h}{max}\right) e^{-c max \left(1 - \frac{h}{max}\right)^2}. \quad (6.9)$$

Crop 3

The third crop had a LAI increasing with height, defined by

$$LAI_h = \begin{cases} \alpha h & \text{for } h \leq max \\ 0 & \text{for } h \geq max \end{cases}$$

So, $\int_0^{\max} LAI_{\sigma} d\sigma = \int_0^{\max} \alpha \sigma d\sigma$ and

$$\lambda_h = l_h \xi 2c \frac{h}{\max} e^{-c(1-\frac{h}{\max})(\max+h)} \quad (6.10)$$

Appendix B

Specified p.d.f.s $D_{n,h}$ for the vertical deposition of spores splashing between two layers n and h both with thickness ξ for the three crop types with LAI distributions as presented in Appendix A.

Crop 1

$$D_{n,h} = \begin{cases} \xi c(1 + e^{-2c(n+a-h)}) e^{-c(h-n)} & \text{if } h > n \\ \xi c e^{-c(2a+n-h)} & \text{if } h < n \\ \xi c(1 + e^{-2ca}) & \text{if } h = n \end{cases}$$

If a spore splashed from a height where $\max < n+a$, then $n+a$ was replaced by \max in the equation where $h > n$; the term $2a+n$ was replaced by $2\max-n$ in the equation where $h < n$ and the term $-2ca$ was replaced by $-2c(\max-h)$ where $h=n$.

Crop 2

$$D_{n,h} = \begin{cases} \xi (1 + e^{-4c(n+a-h-\frac{(n+a)^2-h^2}{2\max})}) e^{-2c(h-n-\frac{h^2-n^2}{2\max})} 2c(1 - \frac{h}{\max}) & \text{if } h > n \\ \xi e^{-ca(2-\frac{a+2n}{\max})} e^{-2c(n+a-h-\frac{(n+a)^2-h^2}{2\max})} 2c(1 - \frac{h}{\max}) & \text{if } h < n \\ \xi (1 + e^{-2ca(2-\frac{a+2n}{\max})}) 2c(1 - \frac{h}{\max}) & \text{if } h = n \end{cases}$$

If a spore splashed from a height where $\max < n+a$, the equations became

$$D_{n,h} = \begin{cases} \xi (1 + e^{-2c(\max-2h+\frac{h^2}{\max})}) e^{-2c(h-n-\frac{h^2-n^2}{2\max})} 2c(1 - \frac{h}{\max}) & \text{if } h > n \\ \xi e^{-c(\max-2n+\frac{n^2}{\max})} e^{-c(\max-2h+\frac{h^2}{\max})} 2c(1 - \frac{h}{\max}) & \text{if } h < n \\ \xi (1 + e^{-2c(\max-2h+\frac{h^2}{\max})}) 2c(1 - \frac{h}{\max}) & \text{if } h = n \end{cases}$$

Crop 3

$$D_{n,h} = \begin{cases} \xi (1 + e^{-\frac{2c}{max}((n+a)^2 - h^2)}) e^{-\frac{c}{max}(h^2 - n^2)} 2ch/max & \text{if } h > n \\ \xi e^{-\frac{c}{max}((n+2a)^2 - h^2)} 2ch/max & \text{if } h < n \\ \xi (1 + e^{-\frac{2c}{max}((n+a)^2 - h^2)}) 2ch/max & \text{if } h = n \end{cases}$$

If a spore splashed from a height where $max < n+a$, then $n+a$ was replaced by max in the equation where $h > n$; the term $(n+2a)^2$ was replaced by $2max^2 - n^2$ in the equation where $h < n$ and the term $(n+a)^2$ was replaced by max^2 where $h = n$.

Chapter 7

General conclusions

This work shows that mechanistic models allow detailed examination of the biological and physical factors influencing the spatial spread of splash dispersed fungal plant pathogens. The main mechanisms underlying splash dispersal are: 1. A raindrop hits the water film formed on a leaf or the ground and spores are dispersed in the splashing rain droplets, and 2. Splashed spores are redistributed in the crop and on the soil surface by secondary splash. These two mechanisms were translated into probabilities and formed the basis in modelling horizontal and vertical spread of splash dispersed pathogens.

Horizontal disease spread

Analysis of a mechanistic random 'jump' model showed that the effects of rain properties and ground cover on the splash process could be seen in their effects on the model parameters. For high rain intensities the probability that a spore was splashed increased and simultaneously more spores were removed from the system. The mean dispersal distance varied according to the size of the splash droplets.

The diffusion approximation for the mechanistic model showed that for splash dispersal a diffusion model is in most situations not a useful approximation. The main problem in using a diffusion model for splash dispersal is that the initial inoculum source is depleted instantaneously at $t=0$. Only in the limit for $t \rightarrow \infty$ does the diffusion model describe the process as well as the mechanistic model. Still we would like to emphasize that during an average rain event spores are dispersed over relatively short distances; this process is best described by the mechanistic approach.

The model was compared to experimental data on spore dispersal from a point source. For this purpose composite variables were derived from the model that are of direct biological relevance. For instance, equations were derived for the total number of spores surrounding the source and distances spores travel during rain events. A description

of the change in total number of spores surrounding the source shows that $N(t)$ first increases from the start of the rain up to $t = -\ln(1-\epsilon)/\lambda\epsilon$, and then declines. $E(r^2)$ gives a good representation of the potential disease spread in a crop, because those spores that travel the larger distances are responsible for disease spread into new areas. Spores will reach their maximum squared distance at $t = 1/\lambda(1-\epsilon)$. A striking result is that the mean-squared displacement of spores is independent of the complicated spore transport distribution as used in the full model. Therefore, it will in many cases be easier to derive and use this quantity than the mean displacement. Testing with experimental data showed that model results were in general agreement with observed data for the rain intensity and ground cover experiments.

Vertical disease spread

An experimental study clearly demonstrated that conidia of *P. brassicae* have the potential to be further dispersed by the action of secondary splash after an initial primary splash from infected plant tissue. The results of these splash experiments suggest that, for practical modelling purposes, "average" trajectories of droplets splashed from oilseed rape plant parts may be approximated by simple parabolic equations. However, the parabola is strongly related to the plant part a spore splashes from, because the structure of plant parts may strongly influence splash droplet trajectories.

These results were incorporated into a model and simulations showed that rain-splash is an important mechanism for the upward disease spread of light leaf spot on winter oilseed rape during the period of stem extension. Disease spreads up the plants by a combination of vertical splash dispersal of spores and stem extension. Weather conditions and crop characteristics influence disease progress particularly at the beginning of stem extension when leaf abscission before a next rain event prevents rapid movement of infectious units to the upper canopy in a crop having a doubled LAI. Periods with frequent rain events in a dense crop canopy (LAI constant with height) were most favorable for disease progress.

The influences of crop characteristics and rain properties on vertical spread was further investigated for three crop types. Splash dispersal was concentrated in the upper canopy layers in a crop having LAI constant or increasing with height. Splash probabilities were greatest and most spores were intercepted in the layers just beneath the upper layers in a crop having LAI decreasing with height.

These results show that both horizontal and vertical spread of splash dispersed diseases in arable crops can be modelled successfully using a mechanistic approach. Model analysis can provide important information on environmental and crop factors potentially influencing disease development and guide further experimental research to ultimately provide important information in developing disease management strategies.

References

- Allen, R.F., 1988. The mechanics of splashing. *Journal of Colloid and Interface Science*, 124:309-316.
- Berrie, A.M., and Luton, M.T., 1996. Integrated control of *Phytophthora* fruit rot *Phytophthora syringae* in stored cox apples. *Proceedings of the Brighton Crop Protection Conference: Pests and Diseases*, 3:925-932.
- Boudreau, M.A. and Madden, L.V., 1995. Effect of strawberry density on dispersal of *Colletotrichum acutatum* by simulated rain. *Phytopathology*, 85: 934-941.
- Carslaw, H.S., and Jaeger, J.C., 1959. *Conduction of Heat in Solids*. Clarendon Press, Oxford.
- Chauhan, V.B., and Singh, U.P., 1991. Effect of weed infestation on the severity of *Phytophthora* blight of pigeon pea. *Plant Disease*, 75:1230-1232.
- Doucet, P., and Sloep, P.B., 1992. *Mathematical modeling in the Life Sciences*. Ellis Horwood Limited, Chichester.
- Edelstein-Keshet, L., 1988. *Mathematical models in Biology*. Random House, New York.
- Evenhuis, A., Verdam, B. and Zadoks, J.C., 1997. Splash dispersal of conidia of *Mycocentrospora acerina* in the field. *Plant Pathology*, 46: 459-469.
- Fatemi, F. and Fitt, B.D.L., 1983. Dispersal of *Pseudocercospora herpotrichoides* and *Pyrenopeziza brassicae* in splash droplets. *Plant Pathology*, 32: 401-404.
- Ferrandino, F.J., and Elmer, W.H., 1996. *Septoria* leaf spot lesion density on trap

plants exposed at varying distances from infected tomatoes. *Plant Disease*, 80:1059-1062.

Figueroa, L., Fitt, B.D.L., Shaw, M.W., McCartney, H.A. and Welham, S.J., 1995. Effects of temperature on the development of light leaf spot (*Pyrenopeziza brassicae*) on oilseed rape (*Brassica napus*). *Plant Pathology*, 44: 51-62.

Fitt, B.D.L., and McCartney, H.A., 1985. Spore dispersal in splash droplets. In: P.G. Ayres and L. Boddy (Editors), *Water, fungi and plants*. Cambridge University Press, Cambridge, pp. 87-104.

Fitt, B.D.L. and McCartney, H.A., 1986. Spore dispersal in relation to epidemic models. In: *Plant Disease Epidemiology; population Dynamics and Management* (ed K.J. Leonard & W.E. Fry) MacMillan Publishing Company. New York, 311-345.

Fitt, B.D.L. and McCartney, H.A., 1986^a. Spore dispersal in splash droplets. In: *Water, Fungi and Plants* (P.G. Ayres and L. Boddy, eds.), pp. 87-104. Cambridge University Press.

Fitt, B.D.L., Creighton, N.F., Lacey, M.E. and McCartney, H.A., 1986. Effects of rain-fall intensity and duration on dispersal of *Rhynchosporium secalis* conidia from infected barley leaves. *Trans. Br. mycol. Soc.*, 86: 611-618.

Fitt, B.D.L., Walklate, P.J., McCartney, H.A., Bainbridge, A., Creighton, N.F., Hirst, J.M., Lacey, M.E., and Legg, B.J., 1986^a. A raintower and wind tunnel for studying the dispersal of plant pathogens by rain and wind. *Annals of Applied Biology*, 109: 661-671.

Fitt, B.D.L., Gregory, P.H., Todd, A.D., McCartney, H.A., and Macdonald, O.C., 1987. Spore dispersal and plant disease gradients; a comparison between two empirical models. *Phytopathology*, 118: 227-242.

Fitt, B.D.L., McCartney, H.A., and Walklate, P.J., 1989. The role of rain in dispersal of pathogen inoculum. *Annual Review of Phytopathology*, 27: 241-70.

Fitt, B.D.L., Inman, A.J., Lacey, M.E., McCartney, H.A., 1992. Splash dispersal of spores of *Pseudocercospora capsellae* (white leaf spot) from oilseed rape leaves of different inclination, flexibility and age. *Journal of Plant Diseases and Protection*, 99: 234-244.

Fitt, B.D.L., Doughty, K.J., Gilles, T., Gladders, P., Steed, J.M., Su, H. and Suther-

- land, K.G. 1998. Methods for assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. *Annals of Applied Biology*, 133: 329-341.
- Fitt, B.D.L., Doughty, K.J., Gladders, P., Steed, J.M. and Sutherland, K.G. 1998^a. Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. *Annals of applied Biology*, 133: 155-166.
- Freitas, M.A., Filho, A.C.C., and Nasser, L.C.B., 1998. Dispersion of soybean stem canker, caused by *Diaporthe phaseolorum f.sp. meridionalis* from a point-source inoculum. *Fitopatologia Brasileira*, 23:161-165.
- Gregory, P.H., 1961. The microbiology of the atmosphere. Leonard Hill [books] limited Interscience Publishers, New York.
- Habekotté, B., 1996. Winter oilseed rape; analysis of yield formation and crop type design for higher yield potential. PhD Thesis. Wageningen Agricultural University.
- Huber, L., McCartney, H.A. and Fitt, B.D.L. , 1997. Influence of target characteristics on the amount of water splashed by impacting drops. *Agricultural and Forest Meteorology*, 87:201-211.
- Inman, A.J. and Fitt, B.D.L., 1992. Epidemiology in relation to control of white leaf spot (*Mycosphaerella Capsellae*) on oilseed rape. Brighton Crop Protection Conference, Pest and Diseases, 681-686.
- Inman, A.J., Fitt, B.D.L., Todd, A.D. and Evans, R.L., 1999. Ascospores as primary inoculum for epidemics of white leaf spot (*Mycosphaerella capsellae*) in winter oilseed rape in the UK. *Plant Pathology*, 48: 308-319.
- Jeffery, D.C., Gareth Jones, D. and Jenkins, P.D., 1989. Effects of early infection of light leaf spot (*Pyrenopeziza brassicae*) on oilseed rape (*Brassica napus*). *Aspects of Applied Biology*, 23: 409-414.
- Jeffery, D.C., Jenkins, P.D. and Gareth Jones, D., 1994. Comparative studies of light leafspot (*Pyrenopeziza brassicae*) epidemics on the growth and yield of winter oilseed rape. *Annals of Applied Biology*, 124: 19-25.
- Johnson, N.L., and Kotz, S., 1970. Laplace Distribution. In: R.A. Bradly *et al.* (Editors), Wiley series in probability and mathematical statistics. John Wiley & sons, pp.22-36.

- Linders, E.G.A., van Damme, J.M.M., and Zadoks, J.C., 1996. Transmission and over-seasoning of *Diaporthe adunca* on *Plantago lanceolata*. *Plant Pathology*, 45:59-69.
- Lovell, D.J., Parker, S.R., Hunter, T., Royle, D.J. and Coker, R.R., 1997. Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. *Plant Pathology*, 46: 126-138.
- Macdonald, O.C., and McCartney, H.A., 1987. Calculation of splash droplet trajectories. *Agricultural and Forest Meteorology*, 39: 95-110.
- Macdonald, O.C. and McCartney, H.A., 1988. A photographic technique for investigating the splashing of water drops on leaves. *Annals of Applied Biology*, 113: 627-638.
- Madden, L.V., and Ellis, M.A., 1990. Effect of ground cover on splash dispersal of *Phytophthora cactorum* from strawberry fruits. *Phytopathology*, 129:170-174.
- Madden, L.V., 1992. Rainfall and the dispersal of fungal spores. *Advances in Plant Pathology*, 8: 39-79.
- Madden, L.V., Wilson, L.L., Yang, X., and Ellis, M.A., 1992. Splash dispersal of *Colletotrichum acutatum* and *Phytophthora cactorum* by short-duration simulated rains. *Plant Pathology*, 41:427-436.
- Madden, L.V., Wilson, L.L., and Ellis, M.A., 1993. Field spread of anthracnose fruit rot of strawberry in relation to ground cover and ambient weather conditions. *Plant Disease*, 77: 861-866.
- Madden, L.V., Yang, X., and Wilson, L.L., 1996. Effects of rain intensity on splash dispersal of *Colletotrichum acutatum*. *Phytopathology*, 86: 864-874.
- Madden, L.V., 1997. Effects of rain on splash dispersal of fungal pathogens. *Canadian Journal of Plant Pathology*, 19: 225-230.
- McCartney, H.A. and Fitt, B.D.L., 1986. Spore dispersal gradients and disease development. Wolfe M.S., and Caten, C.E. *Populations of Plant Pathogens: their Dynamics and Genetics*. Blackwell Scientific Publications, Oxford: 109-118.
- McCartney, H.A. and Lacey, M.E., 1990. The production and release of ascospores of *Pyrenopeziza brassicae* on oilseed rape. *Plant Pathology*, 39: 17-32.

- Mendham, N.J., and Salisbury, P.A., 1995. Physiology: crop development, growth and yield. Kimber, D.S., and McGregor, D.I. *Brassica Oilseed*; production and utilization. University Press, Cambridge: 11-64.
- Mood, A.M., Graybill, F.A., and Boes, D.C., 1950. Introduction to the theory of Statistics. McGraw-Hill Inc., Singapore.
- Morrison, M.J., Stewart, D.W. and McVetty, P.B.E., 1992. Maximum area, expansion rate and duration of summer rape leaves. *Can. J. Plant Science*, 72: 117-126.
- Ntahimpera, N., Ellis, M.A., Wilson, L.L., and Madden, L.V., 1997. Alteration of rain effects on splash dispersal of *Colletotrichum acutatum* by a cover crop. *Phytopathology*, 87:S70 (Abstr.).
- Ntahimpera, N., Madden, L.V. and Wilson, L.L., 1997^a. Effect of rain distribution alteration on splash dispersal of *Colletotrichum acutatum*. *Phytopathology*, 87: 649-655.
- Ntahimpera, N., Wilson, L.L., Ellis, M.A. and Madden, L.V., 1999. Comparison of rain effects on splash dispersal of three *Colletotrichum* species infecting strawberry. *Phytopathology*, 89: 555-563.
- Okayama, K., (1994). Effect of barrier on rain splash dispersal of *Glomerella cingulata* (em *Colletotrichum gloeosporioides*) from infected strawberry plant. *Annals of the Phytopathological Society of Japan*, 60:113-118.
- Othmer, H.G., Dunbar, S.R., and Alt, W., 1988. Models of dispersal in biological systems. *Journal of Mathematical Biology*, 26: 263-298.
- Pielaat, A. and van den Bosch, F., 1998. A model for dispersal of plant pathogens by rainsplash. *IMA Journal of Mathematics Applied in Medicine and Biology*, 15: 117-134.
- Pielaat, A., Madden, L.V. and Gort, G., 1998. Spores splashing under different environmental conditions: a modeling approach. *Phytopathology*, 88: 1131-1140.
- Ramachandran, N., Sarma, Y.R., and Anandaraj, M., 1990. Vertical progression and spread of *Phytophthora* leaf infection in black pepper in areca-black pepper mixed cropping system. *Indian Phytopathology*, 43:414-419.

- Reynolds, K.M., Bulger, M.A., Madden, L.V., and Ellis, M.A., 1987. New methods using simulated rain to study the splash dispersal of plant pathogens. *Phytopathology*, 77: 921-926.
- Reynolds, K.M., Madden, L.V., Reichard, D.L., and Ellis, M.A., 1989. Splash dispersal of *Phytophthora cactorum* from infected strawberry fruit by simulated canopy drip. *Phytopathology*, 79: 425-432.
- Rommelzwaal, A.J. and Habekotté, A., 1986. Simulatie van de fenologische ontwikkeling van winterkoolzaad in de IJsselmeerpolders. Rijp-rapport. Lelystad: 22p.
- Seber, G.A.F., and Wild, C.J., 1989. Nonlinear Regression. John Wiley and Sons, Inc. New York. pp. 768
- Shaw, M.W., 1987. Assessment of upward movement of rain splash using a fluorescent tracer method and its application to the epidemiology of cereal pathogens. *Plant Pathology*, 36: 201-213.
- Sneddon, I.N., 1972. The use of integral transforms. McGraw-Hill Inc., New York.
- Soleimani, M.J., Deadman, M.L. and McCartney, H.A., 1996. Splash dispersal of *Pseudocercospora herpotrichoides* spores in wheat monocrop and wheat-clover bicrop canopies from simulated rain. *Plant Pathology*, 45: 1065-1070.
- Stinchcombe, G.R., Hutcheon, J.A. and Jordan, V.W.L. 1986. Effects of growth regulators on light leaf spot and yield components in oilseed rape. British crop protection conference-Pests and diseases, 1009-1015.
- Stobart, R.M., Simpson, M.J.A., and Cullum, F.J., 1999. Aspects of cultural and chemical control of the pathogen *Septoria oenotherae* in evening primrose (*Oenothera spp.*). *Plant Varieties and Seeds*, 12:53-63.
- Su, H., Fitt, B.D.L., Welham, S.J., Sansford, C.E. and Sutherland, K.G., 1998. Effects of light leaf spot (*Pyrenopeziza brassicae*) on yield of winter oilseed rape (*Brassica napus*). *Annals of Applied Biology*, 132: 371-386.
- Sylvester-Bradley, R., 1985. Revision of a code for stages of development in oilseed rape (*Brassica napus* L). *Aspects of Applied Biology*, 10: 395-400.
- Tayo, T.O. and Morgan, D.G., 1975. Quantitative analysis of the growth, develop-

ment and distribution of flowers and pods in oilseed rape (*Brassica napus* L.). *Journal of Agricultural Sciences*, 85: 103-110.

Walklate, P.J., McCartney, H.A. and Fitt, B.D.L., 1989. Vertical dispersal of plant pathogens by splashing, Part II: experimental study of the relationship between rain-drop size and the maximum splash height. *Plant Pathology* 38: 64-70.

Yang, X., Madden, L.V., Wilson, L.L., and Ellis, M.A., 1990. Effect of surface topography and rain intensity on splash dispersal of *Colletotrichum acutatum*. *Phytopathology*, 80: 1115-1120.

Yang, X., Madden, L.V., and Brazee, R.D., 1991. Application of the diffusion equation for modelling splash dispersal of point-source pathogens. *New Phytologist*, 118: 295-301.

Yang, X., Madden, L.V., Reichard, D.L., Fox, R.D., and Ellis, M.A., 1991^a. Motion analysis of drop impaction on a strawberry surface. *Agricultural and Forest Meteorology*, 56: 67-92.

Yang, X.B., and TeBeest, D.O., 1992. Rain dispersal of *Colletotrichum gloeosporioides* in simulated rice field conditions. *Phytopathology*, 82:1219-1222.

Yang, X. and Madden, L.V., 1993. Effect of ground cover, rain intensity and strawberry plants on splash of simulated raindrops. *Agricultural and Forest Meteorology*, 65: 1-20.

Samenvatting

Spatten, -De verspreiding van schimmelziekten in gewassen tijdens regen-

Spatten is een belangrijk mechanisme voor de ruimtelijk verspreiding van infectieuze deeltjes in een gewas (Madden, 1992). Veel gewassen, groente en fruit, hebben jaarlijks te lijden van infecties waarbij regen zelfs de voornaamste bron voor expansie is. In het Engels wordt dit verspreidingsmechanisme kort maar krachtig aangeduid met de term "splash dispersal" (spatverspreiding). Dit fenomeen is de oorzaak van veel ernstige bovengrondse epidemieën in gewassen in zowel gematigde als tropische klimaten. Toch wordt deze manier van verspreiding vaak niet herkend als het, op wind, één na belangrijkste verspreidingsmechanisme voor veel plantenziekten (Fitt *et al.*, 1989). Symptomen van deze schimmelziekten zijn herkenbaar aan de lesies gevormd op het bladoppervlak. Al snel na aanvang van de regenbui laat de schimmel zijn infectieuze deeltjes (sporen) los uit hun beschermend omhulsel en komen de sporen vrij in het waterlaagje te liggen dat zich op het bladoppervlak gevormd heeft. Als een regendruppel deze waterfilm raakt spat de film uiteen en vormt een kroon van vele kleine druppeltjes. Sporen zijn opgenomen in deze wegspringende druppeltjes en zo verspreid naar andere plaatsen in het gewas (Fitt en McCartney, 1985). Op deze nieuwe plek kan zich een nieuwe infectie ontwikkelen of er kan een nieuwe spatgebeurtenis plaatsvinden waardoor de spore zich verder in het gewas verplaatst. De totale afstand die een spore in meerdere sprongen tijdens een regenbui kan afleggen vanaf de infectiebron hangt niet alleen af van de regenintensiteit en de duur van de regenbui, maar ook van de schimmelsoort, grondsoort en gewasstructuur (Fitt *et al.*, 1992; Madden *et al.*, 1993; Yang en Madden, 1993). De ziekte wordt vaak geïntroduceerd met door de schimmel geproduceerde ascosporen die in de herfst met de wind het gewas binnenkomen. De bron van deze infectie betreft voornamelijk plantafval dat op het veld is blijven liggen nadat het gewas geoogst is. In het gewas vindt daarna geleidelijke horizontale en verticale verspreiding plaats met door spatten verspreide conidiosporen (Inman *et al.*, 1999).

In laboratoria over de hele wereld worden spat-proeven uitgevoerd om dit versprei-

dingsmechanisme te doorgronden met als uiteindelijk doel; bestrijding van deze vorm van ziekteverspreiding. Vaak wordt een regensimulator gebruikt om het spatproces te bestuderen, en worden de effecten van regen, gewas, grond en ziekteëigenschappen op de uiteindelijke verspreiding bepaald (Fitt *et al.*, 1986; Madden *et al.*, 1996; Reynolds *et al.*, 1987; Yang *et al.*, 1991). Veel datasets zijn beschikbaar met informatie over de ruimtelijke verspreiding van zwarte-vlekkenziekte en leerrot in aardbeien en over de verspreiding van bruine-vlekkenziekte in winterkoolzaad. Andere belangrijke ziektes met door spatten verspreide infectieuze deeltjes zijn oogvlekkenziekte, gele en bruine roest op tarwe, bladvlekkenziekte en kafjesbruin op wintertarwe, bladvlekkenziekte op wintergerst, rode kafschimmel op granen, zwartnervigheid op kool, papiervlekkenziekte op prei, bacteriekanker op citrus, en schurft op appels. Echter, ook al worden er nog zoveel experimenten gedaan, het aantal zal nooit toereikend zijn om de ruimtelijke verspreiding onder invloed van de immense variteit in zowel biologische- als omgevingsfactoren te kunnen kwantificeren voor deze zeer uiteenlopende plant/ziekte systemen. De oplossing voor dit probleem wordt gezocht in de modellenwereld. Daarvoor zijn fysische modellen nodig waarin de mechanismen die ten grondslag liggen aan het spatproces worden opgenomen in de vorm van kansfuncties, d.w.z. *i.* een kans per tijdseenheid dat een regendruppel een te infecteren plek in het gewas raakt tijdens de regenbui; *ii.* de kans dat een schimmelspore wordt opgenomen in de wegspringende regendruppeltjes, en *iii.* een kansfunctie die beschrijft waar in het gewas weggesprongen schimmelsporen terecht zullen komen. Met deze modellen kan de ruimtelijke verspreiding van sporen in relatief korte tijd in verschillende plant/ziekte systemen onderzocht worden. Factoren welke het spatproces beïnvloeden zijn nu namelijk als parameters in de kansfuncties van de modellen opgenomen. Door de waarden van deze parameters te veranderen kan de ruimtelijke verspreiding van sporen onder talloze verschillende condities onderzocht worden. Echter, mechanistische modellen kunnen nooit een realistische ziekteverspreiding voorspellen zonder realistische parameterwaarden. Parameterwaarden voor de te onderscheiden processen kunnen verkregen worden uit experimenten. Zodra realistische parameterwaarden zijn opgenomen in het model kunnen de belangrijkste factoren die de ziekteverspreiding beïnvloeden onderzocht worden wat weer kan leiden tot nader experimenteel onderzoek naar deze kernfactoren. Met deze filosofie zijn verschillende mechanistische modellen ontwikkeld om de verspreiding van plantenziekten door regen zowel in ruimte als tijd te onderzoeken. Er zijn experimenten uitgevoerd, maar ook eerder gepubliceerde data gebruikt om realistische parameterwaarden voor de modellen bepalen. De modellen zijn getest met laboratorium- en velddata voor verschillende plant/ziekte systemen.

In hoofdstuk twee wordt een één-dimensionaal mechanistisch "random jump" model geïntroduceerd om de horizontale verspreiding van plantenziekten vanuit een puntbron op de grond te onderzoeken. Grondbedekking en regenintensiteit bleken een grote invloed te hebben op de verspreiding. In zware regenbuien werden meer sporen verspreid, maar tegelijkertijd kwamen er ook meer sporen op plaatsen terecht waarvandaan ze niet

meer verder verspreid konden worden (bijvoorbeeld onder een strolaag). Dit model is vergeleken met een eerder gepubliceerd diffusiemodel door het mechanistische model met een diffusievergelijking te benaderen. Waar het eerder gepubliceerde diffusiemodel uit samengestelde parameters bestaat, was het nu mogelijk deze parameters op te splitsen in onderdelen van de onderliggende biologische processen. Daarnaast is aangetoond onder welke condities het diffusiemodel een geldige benadering voor het mechanistische model is.

In het derde hoofdstuk is de twee-dimensionale versie van het model voor horizontale verspreiding bediscussieerd. Twee biologisch interessante maten zijn afgeleid van dit model. Ten eerste is een vergelijking voor de verandering in het totale aantal sporen in het oppervlak rond de bron sinds de start van de regenbui afgeleid. Daarnaast is een vergelijking voor de gemiddelde en gemiddeld gekwadrateerde afstand die sporen afleggen vanaf de puntbron bepaald. Het model is getest door de resultaten te vergelijken met eerder gepubliceerde experimentele resultaten waarin de verspreiding van conidia vanaf een puntbron (bestaande uit een op de grond geplaatste aardbei) in relatie tot grondsoort en regenintensiteit is onderzocht. Hieruit bleek dat zowel het totale aantal sporen in het proces als de gemiddeld gekwadrateerde afstand aanvankelijk toenam tot een maximum was bereikt en daarna weer afnam doordat sporen op plekken terecht kwamen vanwaar ze niet meer verder konden springen en door bronuitputting.

Niet alleen horizontale, maar ook verticale ziekteverspreiding naar de bovenste bladeren en zaaddragende organen is een belangrijk mechanisme dat schadelijke epidemieën veroorzaakt in wintergewassen in Europa. De ontwikkeling van bijvoorbeeld houttjes in koolzaad en aren in granen bepaalt de opbrengst van deze gewassen. Infectie wordt geïnitieerd in de herfst- en winterperiode en wordt vervolgens opwaarts verspreid door stengelstrekking en regenverspreiding in de lente (Inman en Fitt, 1992; Inman *et al.*, 1999). Dit fenomeen was opgenomen in een simulatiemodel met bruine-vlekkenziekte in winterkoolzaad als modelsysteem. Experimenten zijn uitgevoerd om realistische parameterwaarden te verkrijgen voor dit model en opgenomen in hoofdstuk vier. In een regentoren is het percentage sporen dat opgenomen wordt in de van een blad wegspattende regendruppeltjes bepaald in relatie tot regenduur en grootte van de vallende regendruppels. Daarnaast is de "gemiddelde" ballistische baan die spatdruppeltjes afleggen geschat uit experimenten. Hierin viel een enkele regendruppel op een bepaald plantendeel of op de grond waarna de afstand van de wegspringende druppeltjes t.o.v. de bron werd bepaald. Resultaten lieten zien dat de meeste conidia al in de eerste 15 min. van een regenbui verspreid waren. De ballistische baan die sporen aflegden hing af van het plantendeel waarvan zij wegspatten. De gemiddelde horizontale afstand nam af met toenemende diameter van de inkomende druppel en de maximale spathoogte varieerde van 0.3 cm voor druppeltjes die van een bloemetje sprongen tot 57 cm voor druppeltjes die van de houttjes sprongen. In het vijfde hoofdstuk is het volledige simulatiemodel beschreven bestaande uit een deterministische gemiddelde plantengroei en spatparameters uit de experimenten

beschreven in hoofdstuk vier. De verticale verspreiding van bruine-vlekkenziekte tijdens de groei van koolzaad werd onderzocht voor verschillende condities betreffende ziekte-initiatie en regenduur. Er werd zowel een nat, met lange en frequente regenbuien, als een droog, waarin het korter en minder vaak regende, groeiseizoen gesimuleerd. Tijdens regenbuien werden sporen vertikaal in het gewas verspreid en naar boven getransporteerd op dagen dat stengelstrekking plaatsvond. Perioden met frequente regenbuien in een gewas met grote dichtheid bleek de meeste ziekteverspreiding op te leveren.

Simulatieresultaten in hoofdstuk vijf lieten zien dat gewasstructuur, naast reeds genoemde biologische- en omgevingsfactoren, een belangrijke factor is die ziekteverspreiding beïnvloedt. De invloed van bladoppervlakverdeling over de hoogte in een gewas op de verticale verspreiding van rondspattende deeltjes is daarom onderzocht en beschreven in hoofdstuk zes. Er is een analytisch model ontwikkeld en de invloed van gewasstructuur en regeneigenschappen op de verticale verspreiding is onderzocht door het specificeren van drie gewastypes en waarden van de regenparameters te variëren. Het spatten bleek geconcentreerd te zijn in de bovenste bladeren in een gewas waarin het bladoppervlak constant was of toenam met de hoogte. In een gewas waarin het bladoppervlak afnam met de hoogte was de kans van een spore het grootste om gespat te worden vanaf plaatsen juist onder de top.

Curriculum vitae

Annemarie Pielaat werd op 10 November 1969 geboren te Breskens. Zij bezocht het Koninklijk Wilhelmina Lyceum te Oostburg van 1982 tot 1988. Na een verblijf van een jaar te Brussel ging zij in 1989 milieuchemie studeren aan de Rijkshogeschool IJsselland te Deventer met als hoofdvak ecotoxicologie. In 1993 vervolgde zij haar studie aan de Vrije Universiteit van Amsterdam, waar zij in 1995 het doctoraal examen biologie met als afstudeerrichting theoretische biologie behaalde. Vanaf november 1995 was zij onderzoeker in opleiding bij de sectie wiskundige methoden en modellen van de Wageningen Universiteit, wat geleid heeft tot het voor u liggende proefschrift.