AÉROBIOLOGIE COMPARATIVE DE DEUX OOMYCÈTES

par

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SOMMAIRE

Les oomycètes représentent un groupe d'organismes très diversifiés responsables de diverses maladies d'animaux et de plantes. En agriculture le mildiou de la pomme de terre, causé par un oomycète phytopathogène, occasionne à lui seul des pertes économiques mondiales annuelles de 3 à 5 milliards de dollars américains. La lutte contre le mildiou de la pomme de terre et de la laitue, causés respectivement par, *Phytophthora infestans* et *Bremia lactucae*, repose en grande partie sur l'utilisation de fongicides. Cependant, l'apparition de nouvelles souches résistantes au métalaxyl a réduit de manière substantielle l'efficacité des fongicides de synthèse. De plus, les fongicides doivent être appliqués au moment opportun pour être efficaces contre le mildiou. L'approche par modélisation constitue donc une option intéressante pour améliorer la lutte contre les mildious en offrant la possibilité de prédire les périodes à risque et d'améliorer la régie d'application des fongicides. Toutefois, l'évaluation de ces modèles a montré qu'ils manquent de précision et que leur efficacité varie d'une année à l'autre. Ce manque de précision peut s'explique en partie par l'absence de considération de la présence ou de l'abondance de l'inoculum aérien. *P. infestans* et *B. lactucae* produisent un inoculum adapté à la dispersion aérienne. Cependant, il existe un manque de connaissance notable dans la littérature sur l'aérobiologie de ces deux oomycètes phytopathogènes. Un modèle de simulation dynamique qui intègrerait l'information sur l'aérobiologie de l'agent pathogène permettrait de prédire plus efficacement les périodes de risque d'infection par le mildiou.

Ainsi, l'objectif général de ce projet de doctorat était d'améliorer la connaissance sur l'aérobiologie de deux oomycètes (*P. infestans* et *B. lactucae*) dans l'optique de mieux comprendre et prédire les périodes d'infection du mildiou de la pomme de terre et de la laitue.

Dans un premier temps, la distribution spatiotemporelle des spores à l'échelle d'une région de production de monoculture de pommes de terre a été déterminée. Les résultats ont montré que la distribution des spores est hétérogène et l'efficacité des mesures prophylactiques peut être évaluée à l'aide de l'aire sous la courbe de progression de l'inoculum aérien. De plus, l'information dérivée de l'implantation d'un réseau de capteur permet de pondérer le risque d'infection estimé par les modèles prévisionnels.

Dans un deuxième temps, la relation entre la concentration aérienne de spores et l'intensité du mildiou a été établie. Cette relation a permis de définir des seuils d'intervention basés sur la concentration aérienne de spores. Dans le pathosystème de la pomme de terre, le seuil d'intervention varie en fonction des lignées clonales de *P. infestans* alors que dans celui de la laitue, ce seuil ne tient pas compte des lignées clonales de *B. lactucae*. Un outil moléculaire pour le comptage des spores a été développé pour pallier aux inconvénients liés à la quantification des spores au microscope. Les résultats de cette étude ont montré, entre autres, que sous conditions climatiques au Canada deux heures de mouillure sont suffisantes pour l'établissement d'une infection par *B. lactucae,* contrairement aux trois à quatre heures de mouillure proposées dans la littérature.

Dans un troisième temps, un modèle de simulation de la concentration aérienne des spores a été développé. La relation entre le nombre de spores observées à l'heure et le nombre de spores prédit à l'heure est linéaire. Dans plus de 94% des cas, les coefficients de détermination de la régression entre le nombre de spores observées et celui prédit sont supérieurs à 0,7. Même si ce modèle est une première dans la littérature publiée et représente une avancée significative dans l'aérobiologie des oomycètes, il est à améliorer notamment dans l'optique de pouvoir développer un modèle générique comme outil de recherche pour l'étude de l'aérobiologie des

oomycètes phytopathogènes. En effet, l'étape à venir sera d'incorporer ce modèle comme module dans un système de support décisionnel pour prédire efficacement l'apparition des symptômes de mildiou. Les auteurs espèrent que ce modèle constituera la base pour le développement d'un modèle générique pour tous les oomycètes phytopathogènes à dispersion aérienne.

Mots clés : Système de support décisionnel, *Phytophthora infestans*, *Bremia lactucae*, mildiou, efficience contaminatrice, simulation dynamique.

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TABLE DES MATIÈRES

LISTE DES ABRÉVIATIONS

ASM	American Society for Microbiology
ACC	Airborne Conidia Concentration
ASC	Airborne Sporangia Concentration
ArcGIS	Environmental Systems Research Institute, Inc
Cov	Covariance
CAS	Concentration aérienne de sporanges
DPPCU	Department of Plant Pathology of Cornell University
DSS	Decision Support System
EIPC	Exogenous internal positive control
FPW	Forest *Phytophthora* of the World
GPS	Global Positioning System
G	Grossissement microscope
HSD	Honestly significant difference
INRA	Institut National de la Recherche Agronomique
IPE	Île du Prince Édouard
IE	Infection efficiency
LWD	Leaf wetness duration
NB	Nouveau Brunswick
PCR	Polymerase Chain Reaction
qPCR	Quantitative PCR
pH	potentiel Hydrogène
SSD	Système de Support Décisionnel
Sp	Sporange
SADIE	Spatial analysis by distance indices
SS	Seuil de Signification
R^2	Coefficient de détermination

β	Le coefficient Bêta
Cm	Centimètre
^{o}C	Degrè celsius
M	Moyenne arithmétique
m	Mètre
m^3	Mètre cube
m/s^{-1}	Mètre par seconde
%	Pourcentage
Sec	Seconde
UV	Ultraviolet
µm	Micromètre
V	Variance
Ɵ	Le coefficient Teta

LISTE DES TABLEAUX

CHAPITRE 1

CHAPITRE 2

CHAPITRE 3

CHAPITRE 4

CHAPITRE 5

LISTE DES FIGURES

CHAPITRE 1

CHAPITRE 2

CHAPITRE 3

CHAPITRE 4

CHAPITRE 5

CHAPITRE 1

INTRODUCTION GÉNÉRALE

Ce projet de doctorat porte sur l'aérobiologie des oomycètes par ailleurs, les principes fondamentaux permettant de définir l'aérobiologie, les oomycètes et l'épidémiologie botanique seront abordés dans ce chapitre.

1.1. Aérobiologie

L'aérobiologie est une discipline scientifique qui étudie le transport des organismes et des particules biologiques dans l'atmosphère. L'aérobiologie considère entre autres la dispersion, la viabilité, le transport et l'infection (infestation) de pollen, spores, virus, champignons, bactéries et autres organismes. Le terme bioaérosol définit les particules biologiques aéroportées constituées d'organismes vivants (WAMIS, s.d.; Hurst *et al.,* 2007).

1.1.1. Dispersion des bioaérosols

La dispersion des bioaérosols dans l'atmosphère dépend du processus de libération spécifique à chaque taxon et des facteurs environnementaux tels que la température, l'humidité et la vitesse du vent. Dans le cas des spores de champignons et d'oomycètes, le relâchement dans l'atmosphère peut se faire par un mécanisme de libération passif ou actif (Hurst *et al.,* 2007).

1.1.2. Mécanisme de libération passif

Le mécanisme de libération passif des spores est souvent sous le contrôle des mêmes forces exercées pour assurer le transport à plus ou moins longue distance. Ces forces sont de deux origines : le vent avec les turbulences qu'il provoque et la chute de gouttes d'eau. Cependant, les insectes, les oiseaux et les humains peuvent aussi être à l'origine de la libération passive (Rapilly, 1991).

1.1.2.1. Le vent

L'intervention du vent se fait par sa vitesse, par les changements brutaux de direction et de vitesse et par les turbulences que le climat engendre. C'est la principale stratégie de libération de l'inoculum des agents pathogènes qui sporulent à partir de conidiophores érigés et des spores non ou peu adhérentes entre elles (Rapilly, 1991; Husrt et al, 2007). La vitesse minimale requise du vent pour déclencher la libération varie selon la morphologie des fructifications (ex : conidiophore), du degré de maturité des spores et du déficit de saturation d'eau dans l'air. Plus l'humidité relative de l'air est basse, plus la vitesse minimale du vent requise est faible (Rapilly, 1991). L'augmentation de la vitesse du vent provoque l'agitation du feuillage entraînant ainsi la libération des spores. Aussi, le profil thermique de la végétation, qui occasionne de la turbulence, peut jouer un rôle déterminant dans la libération et le transport vertical (Hurst *et al.,* 2007).

1.1.2.2. Les gouttes d'eau

Les gouttes d'eau issues du brouillard, de la pluie, de l'irrigation représentent une source d'énergie potentielle lors de leur impact sur une fructification. C'est la stratégie de libération et de dissémination de nombreux parasites (champignons, oomycètes) en particulier ceux dont les conidies ou les ascospores sont contenues dans une gelée mucilagineuse (Lepoivre, 2003). Par exemple, *Colletotrichum* disperse ses conidies par la pluie, les premières gouttes d'eau vont dissoudre la gelée mucilagineuse et les gouttes suivantes assurent la dispersion des conidies (Hurts *et al.,* 2007). Les gouttelettes d'éclaboussures qui rejaillissent après l'impact d'une goutte d'eau entraînent avec elles les spores. La concentration en spores de ces gouttelettes varie en fonction de la force d'impact, des caractéristiques de la surface et de la morphologie des fructifications (Rapilly, 1991).

1.1.3. Mécanisme de libration actif

Les mécanismes de libération actifs permettent de projeter les spores hors de la couche limite de la canopée, dans une zone où les turbulences les prendront en charge. Ces mécanismes sont plus puissants chez les ascomycètes que chez les basidiomycètes et quasi-inexistants chez les oomycètes (Hurst *et al.,* 2007). En effet, les ascospores peuvent être projetées de 0,5 cm à 5 cm (Cooke *et al*., 2006). L'énergie de la projection provient de l'augmentation de la pression au sein de la tunique interne de l'asque. Cette augmentation de pression due à l'hydrolyse du glycogène et à l'absorption d'eau entraîne la rupture explosive de l'asque (Hurst *et al.,* 2007). Même si la libération passive prédomine chez la plupart des oomycètes, la séparation des sporanges de leur support est active. Ainsi chez *Phytophthora infestans* et *Peronospora tabacina*, les mécanismes de torsions et détorsions brutales

des sporangiophores permettent de séparer les sporanges de leur support (Rapilly, 1991).

1.1.4. Viabilité des bioaérosols dans l'atmosphère

La plupart des spores adaptées à la dispersion aérienne sont résistantes aux conditions environnementales. Cependant, elles restent vulnérables aux températures extrêmes et aux radiations ultraviolettes (UV) qui affectent la viabilité et l'infectivité. Ces conditions sont habituellement présentes durant le jour et à de basses altitudes. Dans la haute atmosphère, la diminution de la température aide à préserver et protéger les spores des radiations UV (Hurst *et al.,* 2007).

1.1.5. Transport et dépôt des bioaérosols

Le transport est assuré par le vent qui conditionne, en partie, la vitesse de propagation d'une épidémie. Le transport peut s'effectuer par rapport à l'horizontale ou à la verticale. Le transport horizontal s'effectue en fonction de la vitesse du vent. Une vitesse du vent de 4 m/sec peut transporter jusqu'à 4 m une particule de 200 µm de diamètre. Le transport vertical résulte du gradient thermique et de la turbulence due au mouvement des masses d'air (Rapilly, 1991; Hurst *et al.,* 2007). Les distances de transport sont de l'ordre de quelques mètres à des dizaines voire centaines de mètres pour les spores anémophiles (spores disséminées par le vent) (Aylor, 2003).

Une grande proportion des bioaérosols libérés retombe à des distances relativement proches de la zone d'émission. Ce sont ces retombées qui caractérisent

le gradient de dispersion (Aylor, 2001). Ce dernier représente la décroissance de la quantité d'unités d'infectieuses qui tombent sur une population hôte en fonction de la distance d'éloignement de la source d'émission. La forme de ce gradient est très différente selon les forces qui assurent la libération et le transport en lien avec la taille et la quantité d'inoculum (unité de dissémination) (Rapilly, 1991).

1.2. Les oomycètes

Les oomycètes constituent une classe d'organismes eucaryotes appartenant au règne des Straménopiles (Lamour et Kamoun, 2009). Certains oomycètes ont un mode de vie saprophyte tandis que d'autres sont des parasites d'algues, d'animaux ou de plantes. Dans ce groupe se trouvent certains agents pathogènes responsables de maladies dévastatrices en agriculture telles que le mildiou de la pomme de terre, le mildiou de la laitue, etc. (tableau 1.1) (Fry et Niklaus 2010). Au sens étymologique du mot, oomycètes signifie « œuf de champignon ». À cause de leur nature fongiforme, de leur mode de nutrition par absorption et de leur mode de reproduction, les oomycètes ont longtemps été classés parmi les champignons. Cependant, les analyses phylogéniques modernes, basées sur l'utilisation des régions intergéniques (ITS), ont permis de montrer que les oomycètes sont plus proches des algues que des champignons. En effet, l'étude de la comparaison des génomes a permis de déterminer l'évidence que l'ancêtre des oomycètes était photosynthétique (Lamour et Kamoun, 2009). Le tableau 1.2 résume l'ensemble des traits qui distinguent les oomycètes des champignons.

1.2.1. Caractéristiques des oomycètes

Les oomycètes se caractérisent par un certain nombre de traits morphologiques et structuraux : la prévalence d'hyphes non cloisonnés refermant plusieurs noyaux (hyphes coenocytiques), l'absence de chitine dans la paroi cellulaire qui est formée de composés cellulosiques et de β-1,3 et β-1,6-glucanes et la diploïdie au cours de l'essentiel du cycle de développement (Parker, 2009). Toutefois, l'une des caractéristiques distinctives des oomycètes est la production de zoospores biflagellées (Figure 1.1) contenues dans des sporanges. Les sporanges intercalaires (à l'intérieur d'un filament d'hyphes) ou terminaux peuvent être de diverses formes (Figure 1.2). Pour certaines espèces, les sporanges ont perdu la fonction de produire des zoospores et germent directement pour former un tube germinatif, dans ce cas, ces sporanges sont comparables à des conidies. La forte motilité des zoospores, pouvant se déplacer sur divers média (films d'eau à la surface d'une feuille, l'eau intercalaire dans le sol et dans les milieux hydroponiques), confère aux oomycètes l'adaptabilité à diverses niches écologiques (Lamour et Kamoun, 2009).

Figure 1.1. Zoospore biflagellée
(Tirée de Schumann *et al.*, 2005)

Phytophthora cinnamomi *Hyaloperonospora arabidopsidis* *Phytophthora infestans*

Figure 1.2. Variabilité des formes de sporanges chez les oomycètes (Tirée de Fry *et al.*, 2010)

1.2.2. Classification des oomycètes

Les études phylogéniques ont démontré que les oomycètes appartiennent au supergroupe des Chromalvéoles. Ce dernier est subdivisé en deux groupes, les chromistes (*Chromista*) et les alvéolés (*Alveolata*). Le groupe des chromistes comprend trois phyla : les Cryptophytes, les Hapyophytes et les Straménopiles. Ce dernier contient la classe des oomycètes (NCBI s.d; Lamour et Kamoun, 2009). Selon la classification phylogénique, cette classe comporte 23 familles, 90 genres et plus de 1200 espèces (DPPCU s.d). La plupart des oomycètes parasites des plantes cultivées appartiennent à l'ordre des péronosporales, pythiales et albuginales (Figure 1.3) (Blum *et al.,* 2011).

Tableau 1.1. Exemple de maladies causées par des oomycètes (Adapté de Parker, 2009)

Espèces	Plantes Hôtes	Maladie	Mode de vie
Phytophthora infestans	Pomme de terre et tomate	Mildiou	Hémibiotrophe
Phytophthora sojae	Soja	Fonte des semis et pourritures des racines	Hémibiotrophe
Phytophthora ramorum	Arbres (ex. chêne)	L'encre des chênes	Hémibiotrophe
Phytophthora capsici	Multiples hôtes (Cucurbitacées)	Brulure de feuille	Hémibiotrophe
Albugo candida	Brassicaceous (Moutarde) Arabidopsis thaliana	Rouille blanche	Biotrophe obligatoire
Bremia lactucae	Laitue	Mildiou	Biotrophe obligatoire
Plasmopara viticola	Vigne	Mildiou	Biotrophe obligatoire
Pythium ultimum	Multiple dicotylédone	Fonte des semis	Nécrotrophe
Phytophthora cinnamomi	Plus de 300 espèces	Pourriture racinaire	Nécrotrophe
Aphanomyces euteiches	Légumineuses (Médicago truncatula)	Pourriture racinaire	Nécrotrophe

Tableau 1.2. Comparaison entre oomycètes et champignons (Adapté de DPPCU, s.d)

Caractéristique	Oomycètes	Champignons
Croissance par extension polarisée d'hyphes	Oui	Oui
Reproduction par des spores	Oui	Oui
Biosynthèse de stérol	Non (Péronosporales)	Oui
Production de zoospores	Biflagellés	Non ou monoflagellés
Position flagelle	Antérieure et Postérieure	Postérieure
Composition de la paroi cellulaire	β-1-3 et β-1,6-glucanes; cellulose	Chitine
Stockage des réserves	β-1-3-glucanes	Glycogènes
Cellules somatiques	Coenocytique	Cloisonnées (plupart)
Noyau somatique	Diploïde	Haploïde

L'ordre des péronosporales comprend de nombreuses espèces phytopathogènes responsables de maladies communément appelées mildious: par exemple, *Phytophthora infestans* (pomme de terre), *Bremia lactucae* (laitue), *Plasmopara viticola* (vigne) et *Phytophthora sojae* (soja). *P. infestans* est l'agent pathogène à l'origine de la grande famine du XIXe siècle en Irlande (Skelsey, 2008a; Lamour et Kamoun, 2009).

L'ordre des saprolégniales regroupe surtout des espèces parasites des amphibiens et des poissons (Cordier, 2007). Néanmoins, on dénombre dans cet ordre des espèces parasites de plantes, en particulier le genre *Aphanomyces* regroupant plusieurs espèces de parasites racinaires. Parmi les plus importantes d'un point de vue agronomique, on retrouve *Aphanomyces cochlioides* qui infecte la betterave et l'épinard (Madoui, 2009).

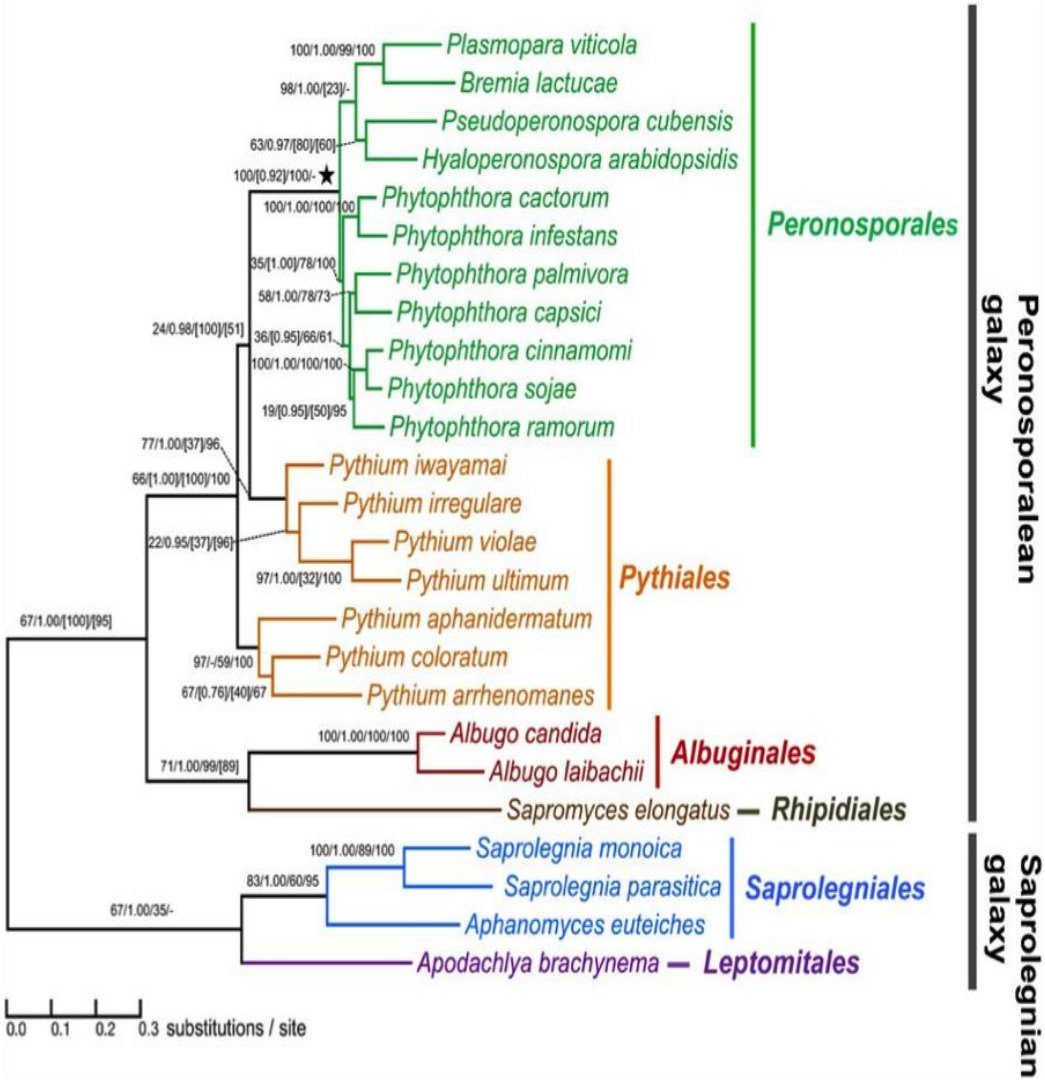


Figure 1.3. Classification des oomycètes parasites de plantes et d'animaux (Blum et al., 2011)

1.2.3. Le cycle de vie des oomycètes

Les oomycètes sont diploïdes sur la grande partie de leur cycle de vie à l'exception de la période de formation des gamètes. La différenciation des hyphes durant la formation des spores permet de lier le cycle sexué et asexué des oomycètes (Figure 1.4) (ASM, 2009).

1.2.3.1. Cycle asexué des oomycètes

La reproduction asexuée des oomycètes comporte principalement deux phases : la sporangiogénèse qui aboutit à la formation de sporanges multinucléés et la zoosporogénèse qui aboutit à la formation de zoospores uninucléées et mobiles. Cependant, certaines espèces d'oomycètes (ex : *P. ramorum* ou *P. cinnamomi* etc.) peuvent produire des spores asexuées appelées chlamydospores (structure de survie plus résistante que le sporange provenant de la fragmentation d'un mycelium) lorsque les conditions environnementales sont défavorables. Ces chlamydospores peuvent se développer en tube germinatif ou en sporanges (Lamour et Kamoun, 2009; Hwang et Ko, 1977). Les sporanges et les zoospores sont les principales propagules de dispersion et constituent le plus important moyen d'initiation d'infection chez les oomycètes pathogènes (Lamour et Kamoun, 2009).

Sporangiogenèse. La sporulation asexuée est dictée par les conditions qui restreignent la croissance, en particulier la disponibilité des nutriments. Mais, elle est aussi dépendante de plusieurs facteurs environnementaux (température, luminosité, humidité, sources de carbones et d'azote et d'ions inorganiques) (Lamour et Kamoun, 2009). La sporangiogénèse implique la différentiation des hyphes pour former des

sporanges intercalaires ou terminaux ou pour former des sporangiophores portant les sporanges (Lamour et Kamoun, 2009; Cordier, 2007). Les sporanges intercalaires ou terminaux ont tendance à rester attachés aux mycéliums tandis que les sporanges issus des sporangiophores peuvent se détacher plus facilement pour devenir des propagules dispersives (Cordier, 2007). La morphologie des sporangiophores et des sporanges varie chez les oomycètes, par exemple, il existe quatre types de sporangiophore chez le genre *Phytophthora* (Figure 1.5) (Cordier, 2007).

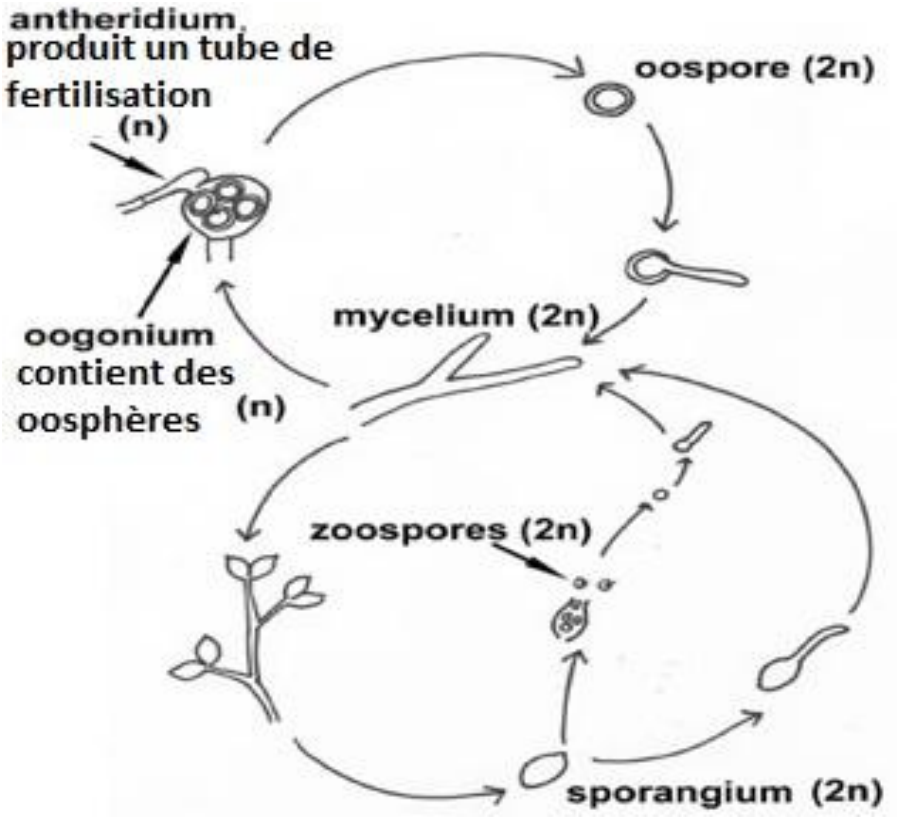
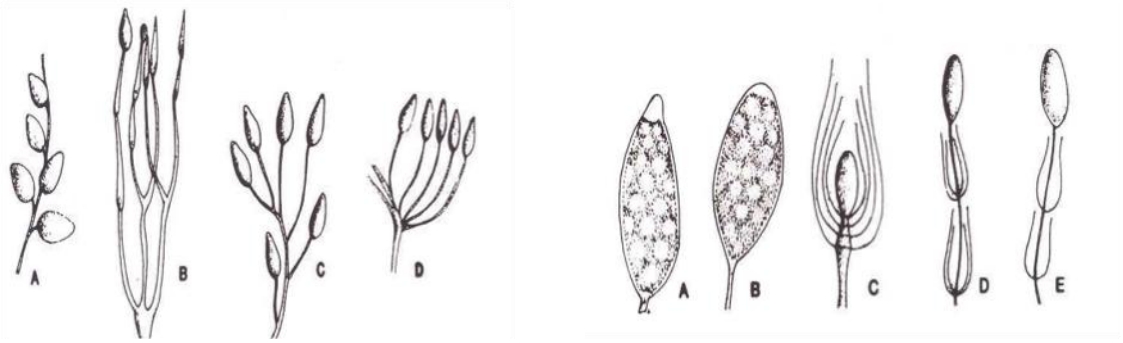


Figure 1.4. Cycle de vie des Oomycètes (Tirée de DBAU, s.d)

La mitose s'effectue dans les sporanges qui peuvent contenir 2, 4, 8 ou 16 noyaux. Toutefois, la plupart des noyaux sont transportés de l'hyphe vers le cytoplasme des sporanges (Lamour et Kamoun, 2009; Marks, 1965). La paroi cellulaire des sporanges est plus épaisse que celle des hyphes somatiques et elle est imperméable aux molécules extracellulaires en solutions (Lamour et Kamoun, 2009).



Morphologies des sporangiophores
A, simple sympode, **B**, sympode caduque
C, sympode composé, **D**, parapluie

Morphologies des sporanges
A, papillé, **B**, semi papillé, **C**, nidifiant
D, nidifiant extensif, **E**, extensif

Figure 1.5. Morphologie des sporangiophores et des sporanges chez les oomycètes (Tirée de Cordier, 2007)

Zoosporogénèse. La différenciation des sporanges en zoospores uninucléées est provoquée par des conditions froides et humides. Les changements du pH et de la concentration de calcium représentent les signaux de transduction induisant la différenciation des sporanges (Lamour et Kamoun, 2009). Le mécanisme largement proposé soutient qu'une formation successive de cloisons entre les noyaux précède la formation des zoospores. La morphologie et le contenu cytoplasmique des zoospores sont identiques chez tous les oomycètes (Lamour et Kamoun, 2009). Les zoospores portent deux flagelles de longueurs différentes. Le flagelle antérieur est effilé, plus long et possède des mastigonèmes (cils) tandis que, le flagelle postérieur n'est pas effilé et ses mastigonèmes sont moins longs (Cordier, 2007).

1.2.3.2. Cycle sexué des Oomycètes

Deux formes de reproduction sexuée, homothallique et hétérothallique, existent chez les oomycètes. Les oomycètes homothalliques peuvent engager la reproduction sexuée en absence de partenaire (autofertilisation) alors que la reproduction sexuée chez les hétérothalliques nécessite la présence de types sexués distincts (mâle et femelle). Par exemple, les types sexués sont A1 et A2 pour *Phytophthora infestans* et B1 et B2 pour *Bremia*. Certaines espèces hétérothalliques sont mâles ou femelles alors que d'autres sont bisexuées (Lamour et Kamoun, 2009). La reproduction sexuée commence par la production de gamètes: oogone et anthéridie (Figure 1.6) qui vont former une oospore (Fry et Niklaus, 2010). L'oogone se forme sans cloisonnement le séparant du reste du thalle contrairement à l'anthéridie qui est délimité par des cloisons. L'origine et l'orientation des gamètes (anthéridie et oogone) sont très diversifiées. Ils peuvent provenir d'un même et unique thalle ou de thalles distincts. Les gamètes se développent en position terminale, subterminale ou intercalaire du thalle (Lamour et Kamoun, 2009). L'appariement entre l'anthéridie et l'oogone est facilité par un adhésif secrété par l'anthéridie. La fécondation de l'oogone se fait de deux manières selon les espèces. La fécondation dite paragyne où l'anthéridie féconde l'oogone latéralement et la fécondation dite amphigyne où l'anthéridie encercle l'oogone au niveau du pore de fertilisation (Figure 1.6) (Lamour et Kamoun, 2009; Cordier, 2007). L'oogone fertilisée se développe en une oospore à paroi épaisse dont le patron de développement est variable selon l'ordre. Par exemple, les oomycètes appartenant à l'ordre des Péronosporales produisent une seule oospore comparativement à certains oomycètes de l'ordre des Saprolégniales qui produisent plusieurs oospores (Fry et Niklaus, 2010; Lamour et Kamoun, 2009). Les oospores se développent dans les cellules de l'hôte avant de rejoindre le sol et sont capables de s'y maintenir durant l'hiver et de réinfecter de nouveaux hôtes (Montarry, 2007).

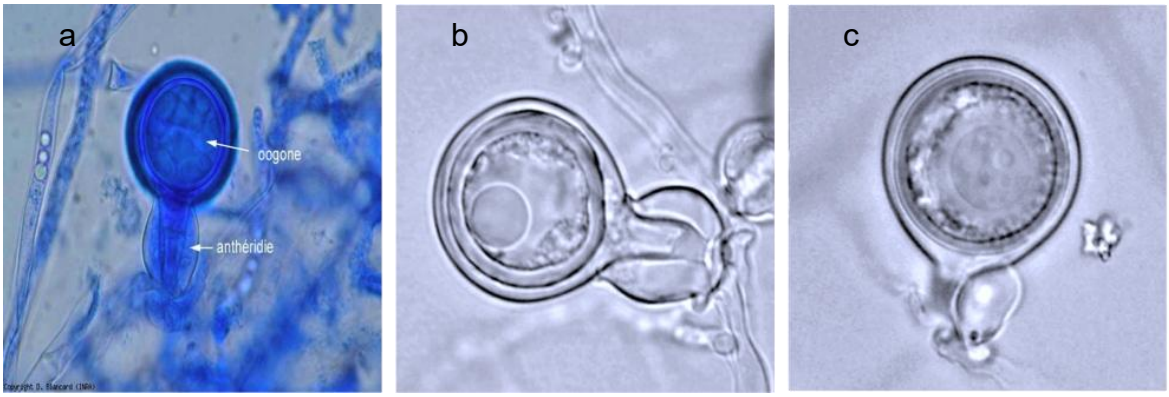


Figure 1.6. Oogone et anthéridie (a), fécondation amphigyne (b) et paragyne (c)(Adaptée de Chamont, 2010; FPW-net, 2012)

1.2.3.3. Régulation du développement sexué

Plusieurs facteurs interviennent dans le développement sexué des oomycètes notamment la nature de l'appareil végétatif (homothallique ou hétérothallique) et leur disposition à générer des gamètes mâles ou femelles ainsi que les conditions environnementales. La production de gamétanges et les étapes subséquentes de l'oosporogénèse sont régulées par des phéromones diffuses anthéridiol (produit par la femelle) et oogoniol (produit par le mâle). Les types sexués se distinguent par la différence dans la perception et la production d'hormones. Ce système est appelé hétérothallisme hormonal où la détection de l'hormone du sexe opposé établit la compatibilité sexuelle. Une fois cette compatibilité établie, les gamètes formés peuvent soit réaliser une autogamie ou une allogamie (selfed and outcrossed coupling) (Lamour et Kamoun, 2009).

1.2.3.4. Importance du cycle sexuel

Dans les systèmes agricoles, l'oospore représente une structure de survie aux conditions environnementales défavorables et une propagule d'infection pour le maintien du succès reproductif (Cordier, 2007). Par leur aptitude à résister aux conditions physicochimiques, les oospores contribuent davantage à la persistance des oomycètes comparativement aux spores asexuées. En effet, la résistance des oospores à la fumigation rend difficile le contrôle des infections en milieu agricole (Lamour et Kamoun, 2009).

Pour certaines maladies causées par oomycètes, l'oospore constitue l'inoculum primaire alors que pour d'autres, ce sont les spores asexuées. Les maladies qui se répandent par les oospores sont généralement monocycliques avec un cycle d'infection par saison, contrairement à celles qui utilisent des spores asexuées pour se propager qui sont généralement polycycliques. Dans ce cas, les oospores et les spores asexuées causent respectivement l'infection primaire et les subséquentes (ex. *Peroscleropora sorghi*) (Lamour et Kamoun, 2009). Un exemple de maladie où l'oospore joue un rôle principal est le mildiou du soja (*P. sojae*) où la plupart des infections primaires sont causées par les oospores qui ont survécu à l'hiver. Les spores asexuées sont produites plus tard dans la saison, mais les infections sont limitées parce que les plantes deviennent résistantes avec l'âge (Lamour et Kamoun, 2009).

1.2.4. Mécanisme d'infection des Oomycètes

Les zoospores d'oomycètes sont attirées à la surface d'un hôte potentiel par la détection du gradient chimique et électrique. Par exemple, les zoospores de *P. sojae* et *Aphanomyces cochlioides* sont attirées par les exsudats racinaires : flavonoïdes et isoflavonoïdes. Cependant, la région cible des zoospores varie d'une espèce de plantes à une autre et d'un agent pathogène à un autre. La nature des récepteurs responsables de la réaction de chimiotactisme et d'électrotactisme n'est pas encore élucidée. Cependant, deux gènes impliqués dans la régulation de la motilité des zoospores ont été identifiés (Lamour et Kamoun, 2009). Une fois à la surface de l'hôte, les zoospores se détachent de leurs flagelles, s'enkystent en élaborant une paroi cellulaire (Figure 1.7) (Cordier, 2007; Lamour et Kamoun, 2009). Cette dernière se prolonge et s'épaissit pour former un appressorium moins différencié que celui de certaines espèces de champignons telles que *Magnaporthe* ou *Colletotrichum* (Lamour et Kamoun, 2009).

Figure 1.7. Zoospores enkystées de *P. sojae* (Tirée de Cordier, 2007)

À l'image de l'appressorium des champignons, l'appressorium des oomycètes est une structure permettant d'exercer une pression à la surface de la plante et de faciliter la pénétration (Grenville-Briggs *et al.,* 2008). L'hyphe d'invagination synthétise et sécrète des enzymes qui vont dégrader les composantes de la paroi cellulaire de la plante telles que la pectine et la cellulose (Grenville-Briggs *et al.,* 2008). La progression de l'hyphe dans la cellule hôte s'effectue par croissance apicale nécessitant de nouvelles composantes membranaires (Lamour et Kamoun, 2009; Parker, 2009).

Suite à la pénétration dans les cellules de l'épiderme, l'hyphe se ramifie dans les tissus de l'hôte par croissance intercellulaire ou intracellulaire. Pour les agents pathogènes nécrotrophes, la colonisation s'accompagne de la mort des cellules de l'hôte. En effet, les sources de nutriments des oomycètes nécrotrophes proviennent des cellules mortes ou des cellules en processus de dégénérescence (Parker, 2009). Les agents pathogènes biotrophes (*Bremia, Plasmopara*, *Hyalperonospora*, etc.) ont besoin de maintenir l'intégrité des cellules de l'hôte pour établir une relation trophique stable avec elle. C'est le cas aussi de la phase initiale biotrophe des espèces hémibiotrophe comme *P. infestans* (Figure 1.8) (Parker, 2009). Les espèces biotrophes obligatoires sont caractérisées par la formation d'un haustorium. Cette dernière est une structure intracellulaire impliquée dans l'acquisition de l'eau et de nutriments déclenchant au même moment la réaction de défense de la plante (Parker, 2009; Lamour et Kamoun, 2009, Lee et al., 2010). En effet, aussi bien dans les interactions compatibles qu'incompatibles, les premières cellules envahies par un oomycète subissent des changements qui sont associés à la défense basale. Chez les plantes hôtes susceptibles, l'inefficacité de cette première réponse entraîne la progression de l'agent pathogène et le développement d'un haustorium (Lamour et Kamoun, 2009). Des protéines effectrices de l'agent pathogène permettent de contrôler la mort cellulaire programmée de certaines cellules de la plante et le transport de nutriments de la plante vers l'haustorium (Figure 1.8) (Lee et al.,

2010). Chez les oomycètes, une interaction compatible aboutit à la sporulation. Des spores asexuées (sporanges, chlamydospores) ou sexuées (oospores) se forment à la surface de la plante et le cycle de l'agent pathogène reprend (Lamour et Kamoun, 2009).

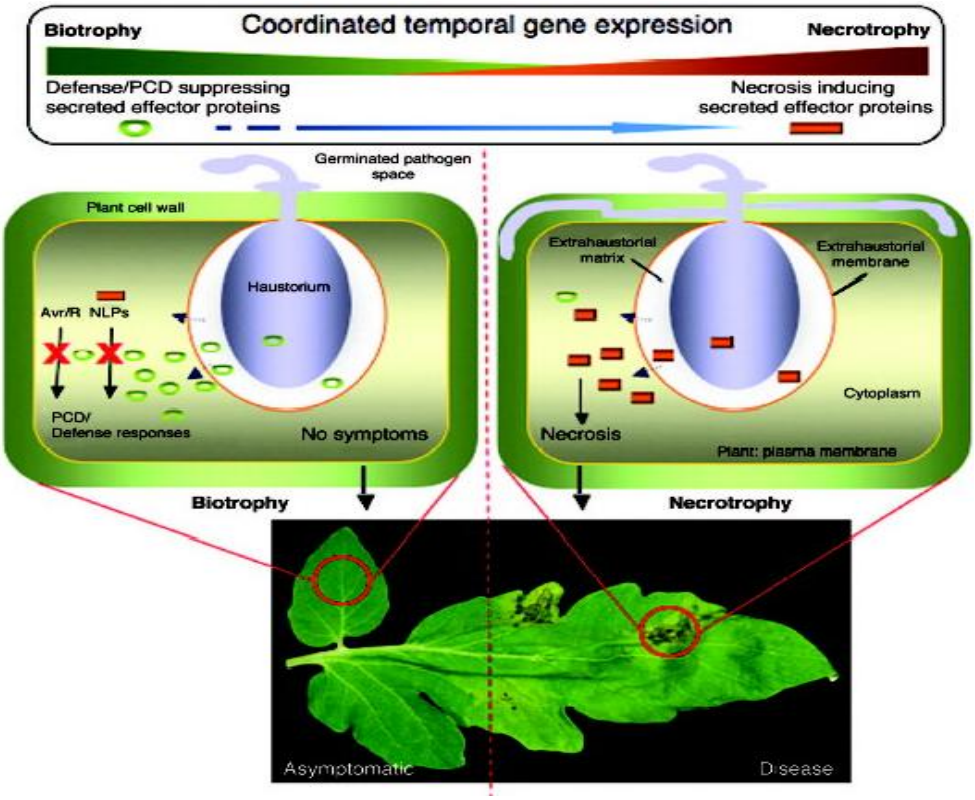


Figure 1.8. Modèle hypothétique de contrôle de l'infection par *Phytophthora infestans*. À droite, la phase biotrophique (feuille sans lésion) et à gauche, la phase nécrotrophique (feuille avec lésion, tache brun foncé ou noire). Les cercles verts représentent les protéines effectrices secrétées par *P. infestans* pour bloquer la mort cellulaire programmée (phase biotrophique). Les rectangles rouges représentent les protéines effectrices secrétées par *P. infestans* pour induire rapidement la mort des cellules végétales (la phase nécrotrophique) (Tirée de Lee et al., 2010).

1.2.5. Exemple d'oomycètes phytopathogènes

Les oomycètes sont parmi les agents phytopathogènes les plus dévastateurs des plantes cultivées, ornementales et forestières (Cordier, 2007). Parmi les maladies de plantes cultivées nécessitant une forte utilisation de produits chimiques, le mildiou de la pomme de terre et le mildiou de la laitue sont parmi les plus importantes. Par exemple, au Canada, l'application de fongicides s'effectue à des intervalles de 7 à 10 jours pour le contrôle du mildiou de la laitue et 80 % des fongicides utilisés au Nouveau-Brunswick et à l'Île-du-Prince-Édouard sont pour lutter contre le mildiou de la pomme de terre (Kushalappa, 2001; Leclerc Yves, communication personnelle, 2011).

1.2.5.1. Phytophthora infestans

Phytophthora infestans de Bary est l'agent pathogène responsable du mildiou de la pomme de terre (Fry & Goodwin, 1997; Aylor *et al.,* 2009). C'est l'une des premières maladies des cultures à avoir été découverte et elle connait une répartition géographique mondiale (Lamour et Kamoun, 2009).

1.2.5.1.1. Distribution géographique et évolution de l'agent pathogène

Pendant de nombreuses années, *P. infestans* a survécu uniquement par multiplication asexuée (par production de sporanges) avec le type sexué A1, sauf dans le centre du Mexique où la coexistence des deux types sexués (A1 et A2) a permis la reproduction sexuée (Lamour et Kamoun, 2009). Cependant, depuis la fin

des années 1980 et le début des années 1990, de nouvelles souches se sont établis presque partout dans le monde (Lamour et Kamoun, 2009). Ces nouvelles souches ont acquis la résistance au métalaxyl, le seul fongicide systémique et curatif largement utilisé depuis 30 ans dans les régions de production de pommes de terre. De plus, elles ont pu contourner la résistance des nouvelles variétés de pomme terre dès leur introduction. Ainsi, *P. infestans* s'est adapté à toutes les mesures antiparasitaires prises depuis 150 ans et reste toujours aussi répandu et nuisible à l'échelle mondiale (Platt, 2008).

1.2.5.1.2. Cycle de vie de l'agent pathogène

Les sporanges de *P. infestans* se forment sur des sporangiophores à la surface du tissu hôte (plantes ou tubercules) (Kirk *et al.,* 2004). Ces sporanges sont adaptés à la dispersion aérienne ou par l'eau. Par ailleurs, ils peuvent se déplacer de 40 à 60 km de la source d'inoculum (Aylor *et al.,* 2001; Platt, 2008). La germination des sporanges en tubes germinatifs est stimulée par une température supérieure à 16°C alors qu'une température inférieure à 16°C entraîne la différenciation de chaque sporange en 8-12 zoospores (Nyankanga *et al.,* 2010; Kirk *et al.,* 2004) (Figure 1.9). Le lessivage des spores produites au niveau des feuilles par la pluie ou l'irrigation, entraîne l'infection des tubercules. Ainsi, *P. infestans* peut survivre d'une année à une autre dans les tubercules en entreposage. La production d'oospores nécessite la présence des deux types sexués (A1 et A2) (Kirk, 2004).

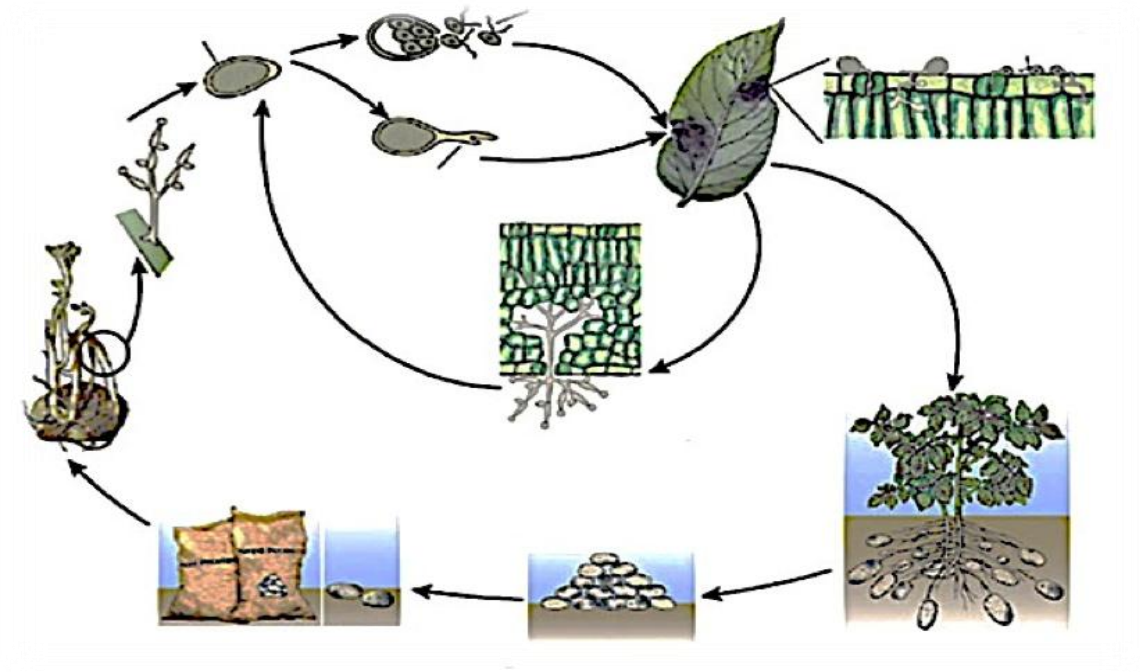


Figure 1.9. Cycle de vie asexué de *P. infestans* en fonction des saisons, Printemps, Été, Automne, et Hiver. Des tubercules de pomme de terre infectés produisent l'inoculum au printemps, les cycles d'infection et de dispersion de l'inoculum maintiennent le développement du mildiou durant l'été, le lessivage des feuilles infectées à l'automne entraîne l'infection des tubercules qui seront entreposés durant l'hiver (Adaptée de Kirk, 2004)

1.2.5.1.3. Développement de la maladie

Une humidité relative supérieure à 90 % et une plage de température optimale de 16° à 21°C stimulent la sporulation et la germination des spores de *P. infestans* (Platt, 2008). Les premiers symptômes du mildiou de la pomme de terre sont de petites lésions, de formes irrégulières à circulaires apparaissant 3 à 5 jours après l'infection (Figure 1.10). Les symptômes apparaissent d'abord sur les feuilles inférieures où le microclimat est plus humide. Le développement de lésions débute souvent dans les

sections de la feuille près du point d'attachement au pétiole (souvent creuses) ou dans les marges des feuilles (Kirk, 2004). Des conditions météorologiques fraîches et humides entraînent la progression des lésions qui deviennent des taches brun foncé ou noires. Des fructifications blanches et veloutées peuvent être observées sur les feuilles et distinguent le mildiou de la pomme de terre des autres maladies de la pomme de terre (Figure 1.10) (Kirk, 2004).

Figure 1.10. Symptômes du mildiou de la pomme de terre, (a) Lésion et structure de croissance blanche et veloutée sur la face inférieure des feuilles, elles peuvent aussi être observées sur la face supérieure des feuilles et sur les tiges (b, c) lorsque les conditions météorologiques sont fraîches et humides (Tirée de Kirk, 2004).

1.2.5.2. Bremia lactucae

Bremia lactucae est l'agent pathogène responsable du mildiou de la laitue. C'est l'une des plus importantes maladies dans la culture de laitue à travers le monde (Kushalappa, 2001; Lamour et Kamoun, 2009). Le mildiou de la laitue peut être très

dévastateur lorsque les conditions climatiques favorisent sa propagation (Carisse et Philion, 2001).

1.2.5.2.1. Distribution géographique et évolution de l'agent pathogène

Bremia lactucae attaque environ 230 plantes de la famille des astéracées, cependant, la seule culture économiquement importante attaquée par *Bremia* est la laitue qui est l'une des 10 cultures les plus importantes aux États-Unis (Kushalappa, 2001; INRA, 2010; Lamour et Kamoun, 2009). Le mildiou de la laitue est l'une des maladies les plus anciennes, les plus fréquentes et les plus redoutables dans la culture de laitue. Il se manifeste surtout dans les zones de production avec des périodes prolongées d'humidité (pluie, irrigation, brouillard, rosée, etc.) et un temps frais. Ainsi, *Bremia* a été répertorié dans la majorité des pays tempérés producteurs de salade (Lamour et Kamoun, 2009; INRA, 2010).

La stratégie mondiale de lutte repose sur l'utilisation de produit chimique (metalaxyl) et de variétés de laitue résistantes. Cependant, les produits chimiques sont devenus peu efficaces pour contrôler le mildiou. La résistance des plants de laitue cultivée est conférée par les gènes Dm qui rendent les plants de laitue résistants à *B. lactucae* jusqu'à l'apparition de nouvelle mutation dans le génome de l'agent pathogène (Lepoivre, 2003; Lamour et Kamoun, 2009).

1.2.5.2.2. Cycle de vie de l'agent pathogène

Les conidiophores émergent à partir des stomates durant la nuit en présence de forte humidité. Le relâchement des conidies est sous le contrôle de l'humidité relative et de la luminosité. En effet, le relâchement des conidies a lieu avant la levée du jour en synergie avec la diminution de l'humidité relative et l'augmentation de la température (Su et al, 1999; Carisse et Philion, 2002).

Les conidies sont relâchées et sont immédiatement entraînées par le vent et les courants d'air. Ainsi, l'infection des plantes voisines ou éloignées s'effectue dans les 3h de mouillure matinale des feuilles (Su *et al.,* 1999; Blancard, 2011). Une température comprise entre 10° et 15°C favorise la germination des conidies et le cycle de l'agent pathogène reprend par une nouvelle sporulation (Figure 1.11). Avec des conditions environnementales favorables *B. lactucae* peut compléter son cycle en 5 jours (Blancard, 2011).

1.2.5.2.3. Développement de la maladie

Les jeunes plants de laitue sont particulièrement sensibles au mildiou et l'infection se fait en 3-4 h en présence de gouttelettes d'eau sur les feuilles (Blancard, 2011; Scherm et van Bruggen, 1994). Les symptômes sont de larges taches vert pâle à jaunes, délimitées par les nervures (Figure 1.12 a) (Blancard, 2011). Ces taches finissent par nécroser et tirent vers une coloration brun clair. La sporulation de *Bremia* débute sur la face inférieure des feuilles avant ou après que les taches chlorotiques soient visibles sur le limbe. Un feutrage blanc plus ou moins dense apparaît sur ces

feuilles (figure 2.12 b). Par la suite, les feuilles fortement touchées nécrosent et meurent (Blancard, 2011).

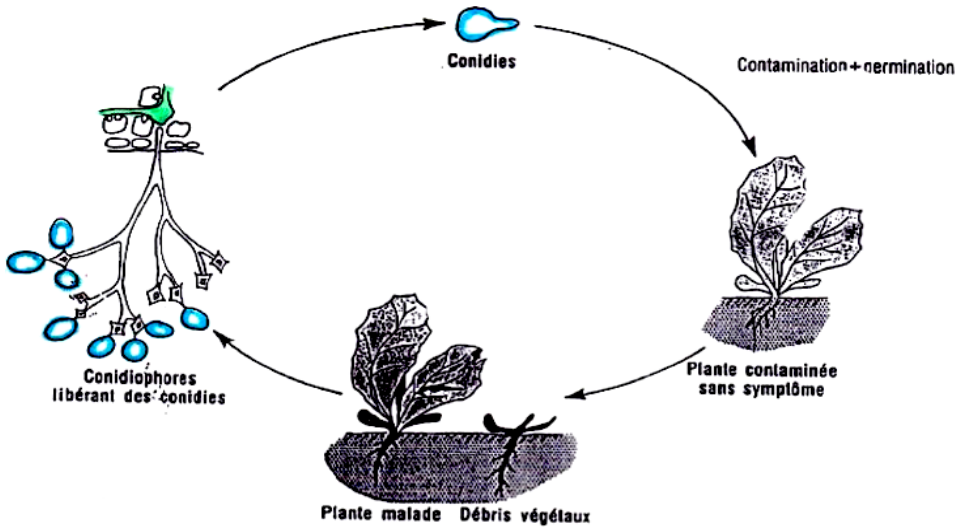


Figure 1.11. Cycle de vie de *Bremia lactucae*. Les conidiophores libèrent les conidies qui sont disséminées, les plantes infectées peuvent être sans symptômes pendant une certaine période de temps (Adaptée de Blancard, 2011)

Figure 1.12. Symptômes du mildiou de la laitue, (a) taches brun clair, (b) feutrage blanc de la sporulation (Tirée de Blancard, 2011)

1.3. L'épidémiologie

L'épidémiologie est une science qui étudie le développement d'une population d'agent pathogène au sein d'une population hôte (Lepoivre, 2003). L'épidémiologie végétale a comme objectif principal d'étudier les maladies de végétaux et la dynamique des populations dans un environnement naturel (Kranz et Rotem 1988). En ce sens, elle fournit par sa démarche les outils nécessaires pour comprendre les relations qui régissent le fonctionnement d'un pathosystème. Ainsi, l'épidémiologie permet d'élaborer des moyens de lutte et d'évaluer leur impact sur le déroulement d'une maladie dans le temps et dans l'espace (Lepoivre, 2003). Du point de vue épidémiologique, une maladie est déterminée par l'interaction tripartite entre un peuplement végétal, une population d'agents pathogènes et l'environnement. Ce triangle de la maladie peut se transformer en tétraèdre avec l'intervention de l'homme en tant que gestionnaire des écosystèmes (Lepoivre, 2003).

1.3.1. La dynamique d'une épidémie

Le développement d'une épidémie représente un enchaînement successif des évènements qui constituent le cycle épidémique de base (Lepoivre, 2003). Ce dernier peut être défini comme un ensemble de compartiments correspondant aux changements d'état de l'agent pathogène et de la maladie qui se succèdent sous l'effet des facteurs environnementaux (Rapilly, 1991). Le cycle élémentaire (monocycle) utilisé en épidémiologie correspond au cycle de développement de l'agent pathogène décrivant les évènements qui s'écoulent entre deux générations successives d'entités infectieuses (spores, cellules bactériennes etc.) (Rapilly, 1991). Lorsque le cycle de l'agent pathogène ne se produit qu'une seule et unique fois pendant le déroulement de l'épidémie, ce dernier est qualifié de monocyclique. Dans

la majorité des cas l'épidémie est causée par une récurrence et une juxtaposition de monocycle, d'où la dénomination d'épidémie polycyclique (Lepoivre, 2003).

1.3.2. Les structures génétiques dans une épidémie

Deux principaux cas caractérisent la relation entre agent pathogène et l'hôte : la relation compatible où la virulence de l'agent pathogène lui permet de se multiplier activement et de coloniser l'hôte; et la relation incompatible où l'avirulence de l'agent pathogène mène à un arrêt précoce de la croissance et de la colonisation subséquente des cellules de l'hôte (Lepoivre, 2003). Ces deux types de relation découlent de l'interaction entre les facteurs de résistance de la plante et les facteurs de virulence de l'agent pathogène. On distingue deux catégories principales de résistance : la résistance verticale caractérisée par des interactions différentielles entre des cultivars différents confrontés à différents pathotypes de l'agent pathogène; et la résistance horizontale caractérisée par l'absence d'interactions différentielles entre les différents cultivars de la plante et les différents pathotype de l'agent pathogène. Tout pathotype de l'agent pathogène qui est capable de surmonter un facteur de résistance verticale est dit virulent (Lepoivre, 2003). En épidémiologie la résistance verticale se manifeste par la réduction de l'inoculum initial qui va engendrer un retard dans le développement de la maladie. Par contre, la résistance horizontale joue sur le taux d'accroissement de l'épidémie et se traduit par un ralentissement de la vitesse de développement de l'épidémie (Van der Plank, 1963).

1.3.3. La mesure des variables épidémiologiques

Les variables épidémiologiques se mesurent sur les composantes du triangle de la maladie (plante, l'agent pathogènes et l'environnement) ou à partir de leur interaction. Ces variables peuvent être l'efficacité contaminatrice, la période de latence, le taux de sporulation, la taille des lésions etc. (Pariaud et al., 2009).

L'efficience contaminatrice peut être définie comme la probabilité qu'une spore déposée sur une hôte susceptible produise une lésion. Elle se mesure habituellement à partir du nombre de lésion obtenu pour un nombre connu de spores déposées (Mehta et Zadoks, 1970). Cependant, elle peut être indirectement mesurée en comptabilisant le nombre de lésions par unité de surface foliaire (Pariaud et al., 2009). Par ailleurs, dans le cadre de projet de doctorat, elle est définie comme étant le rapport entre nombre de lésion par feuille et la concentration aérienne de spores.

La période de latence est l'intervalle de temps entre l'établissement d'une infection et la sporulation de l'agent pathogène. Elle détermine la durée d'un cycle épidémique et la vitesse de développement d'une épidémie (Pariaud et al., 2009). En condition contrôlée, elle représente l'intervalle de temps entre le moment d'inoculation et le premier signe de sporulation (Jeffrey et al., 1962) ou la durée de temps nécessaire pour que 50% des lésions sporulent (T50) ou montrent des symptômes de maladie (Flier et Turkensteen, 1999; Johnson, 1980).

Le taux de sporulation est la quantité de spores produites par lésion et par unité de temps. Il est parfois exprimé en quantité de spores par unité de surface de feuilles malades ou par rapport à la taille des lésion (Pariaud et al., 2009).

La taille des lésions est généralement définie par la surface sporulante. Pour certains agent pathogènes la taille des lésions est limitée alors que pour d'autres comme *Phytophthora infestans* la taille des lésions croit de façon systématique (Pariaud et al., 2009).

1.4. Les statistiques spatiales en épidémiologie

La dimension spatiale est l'un des aspects importants en épidémiologie même si elle est souvent négligée. En effet, la dispersion dans l'espace de l'inoculum ou de la propagule est l'élément clef dans le développement d'une épidémie (Skelsey, 2008a; Gosme, 2007). Elle apporte des informations qui sont importantes à chaque étape d'une étude épidémiologique. Entre autres, elle permet d'estimer l'échelle spatiale pertinente à l'étude d'un pathosystème (Gosme, 2007). Il existe de nombreuses méthodes statistiques permettant d'analyser le développement des épidémies à la fois dans le temps et dans l'espace.

1.4.1. L'analyse spatiale en épidémiologie

La considération de la dimension spatiale d'une épidémie a commencé au cours des années 1980 où de nombreuses méthodes d'analyse de la répartition spatiale des individus ont été développées. L'analyse de la structure spatiale d'une maladie représente la description de la répartition des individus malades dans l'espace et son évolution dans le temps (Gosme, 2007, Carlo et Xavier, 2008). Différentes terminologies sont utilisées pour présenter la répartition dans l'espace des individus malades au sein d'une population de plantes hôtes :

- Agrégation : par exemple, lorsqu'une plante est malade, la probabilité que les plantes voisines soient malades augmente, autrement dit, les individus malades sont regroupés. Dans ce cas, agrégation est synonyme d'hétérogénéité ou de surdispersion, cependant surdispersion réfère à la variance de la distribution de fréquence et non à la répartition spatiale des plantes. L'intensité de l'agrégation représente la saturation relative de la maladie au sein des foyers (Madden *et al.,* 2007).
- Répartition aléatoire : c'est le cas où la probabilité qu'une plante soit malade est indépendante du statut malade des plantes voisines (Madden *et al.,*2007). Autrement dit, chaque point de l'espace a la même chance d'accueillir une plante malade (Gosme, 2007).
- Répartition uniforme : c'est le cas où la probabilité qu'une plante soit malade est réduite par sa proximité à une autre plante malade. Cette situation s'observe rarement dans les études spatiales en phytopathologie (Madden *et al.,* 2007).

L'analyse de la structure spatiale d'une maladie requiert la définition du type de variables considérées. Par exemple, le dénombrement des individus malades dans chaque unité d'échantillonnage permet de définir soit une variable de comptage, si ce nombre n'est pas limité par le nombre d'individus observés, soit une variable d'incidence dans le cas contraire (Gosme, 2007).

1.4.2. Analyse de données non géoréférencées

C'est une analyse basée sur une approche distributionnelle, c'est-à-dire, que l'on teste l'ajustement de la distribution des observations par rapport à une distribution théorique.

Indice de dispersion. Dans le cas des données de comptage, l'indice de dispersion le plus utilisé est le rapport V/M, où V est la variance et M, la moyenne. Un rapport V/M inférieur à 1 indique que la répartition est uniforme, un rapport V/M égal à 1 indique une répartition aléatoire alors qu'un rapport V/M supérieur à 1 indique une agrégation (Carlo et Xavier, 2008). Pour des données d'incidence, l'indice de dispersion est le ratio de deux variances, la variance observée (estimée à partir des données) et la variance estimée sous la prémisse que les données suivent une distribution binomiale (Madden et al, 2007).

Loi de distribution. La répartition spatiale des données est obtenue par la comparaison de la distribution des fréquences observées par rapport à une distribution théorique. Pour des données de comptage, la distribution des fréquences observées est comparée à la distribution de la négative binomiale et à celle de Poisson (Madden *et al.,* 2007). Ainsi, la distribution des fréquences observées suit la loi de Poisson lorsque la répartition est aléatoire et la loi de la négative binomiale lorsqu'il y a agrégation. La loi de la négative binomiale comporte deux paramètres dont k, une mesure d'hétérogénéité. Plus k se rapproche de zéro plus le degré d'agrégation est élevé (Madden *et al.,* 2007).

Pour des données d'incidence, c'est la distribution binomiale et la distribution de la β-binomiale qui servent généralement de base comparative. Quand les fréquences observées suivent la loi de la binomiale, la répartition spatiale des données est aléatoire et quand les fréquences observées suivent la loi de la β-binomiale, on observe une agrégation (Madden *et al.,* 2007). La loi de la β-binomiale fournit deux paramètres dont θ qui reflète l'agrégation, lorsque θ est égal à zéro, on retrouve une répartition aléatoire (Gosme, 2007; Carlo et Xavier, 2008).

Loi de puissance (Taylor). L'agrégation peut être évaluée en utilisant l'analyse par l'ajustement de la loi de puissance qui est liée aux lois de probabilité. La loi de la puissance mise en évidence par Taylor (1961) décrit la relation empirique qui existe entre la variance (V) et la moyenne (M) (Taylor, 1963).

Pour des données de comptage, cette loi s'exprime sous la forme :

$$\ln(V) = \beta_0 + \beta_1 \ln(M)$$

où β_0 et β_1 représentent respectivement l'ordonnée à l'origine (intercept) et la pente de la courbe de régression. Une valeur $\beta_1 > 1$ signifie qu'il y a agrégation dans tous les ensembles de données analysées (Gosme, 2007; Madden *et al.,* 2007).

Pour des données d'incidence, Hughes et Madden (1992) proposent de transformer l'équation précédente sous la forme :

$$\ln(V_{obs}) = \ln(A) + \beta \ln(V_{th})$$

où V_{obs} et V_{th} sont respectivement la variance observée et théorique et ln(A) et β représentent respectivement l'ordonnée à l'origine (intercept) et la pente.

1.4.3. Analyse de données géoréférencées

Ces méthodes d'analyses explicites sont applicables seulement à certains types de données notamment, les données collectées par cartographie intensive. Ce type d'échantillonnage dit spatialisé considère à la fois l'état de la maladie et l'emplacement des unités d'échantillonnage. Toutefois, les analyses de données non géoréférencées sont aussi applicables à ce type de données géoréférencées (Samalens, 2009).

Cartographie. La cartographie permet de représenter la répartition des plantes malades ou de l'intensité de la maladie dans une parcelle sur une carte. En exploitant l'aspect visuel, la cartographie permet d'avoir une vision globale de l'épidémie. Il n'est pas toujours possible d'identifier la structure spatiale d'une épidémie avec de telles cartes. Toutefois, certains logiciels comme SADIE (Spatial analysis by distance indices) ou ArcGIS (Environmental Systems Research Institute, Inc.) permettent d'améliorer la visualisation (Gosme, 2007).

Géostatistique. La géostatistique s'intéresse à la variation d'une variable aléatoire dont les valeurs sont localisées en certains points de l'espace à la condition que les données soient géoréférencées et que la variable soit stationnaire sur l'étendue (surface de la zone d'observation) de l'étude (Samalens, 2009; Carlo et Xavier, 2008). Les calculs de géostatiques sont basés sur la covariance (cov (h)) entre paires de données séparées par une distance h.

La semi-variance quantifie la variance entre les valeurs mesurées à des points séparés par une distance h (cov(0) – cov(h) (Samalens, 2009). Sa représentation

graphique est appelée semi-variogramme (ou variogramme si l'on multiplie cette valeur par deux). La représentation graphique du rapport cov(h)/cov(0) s'appelle autocorrélogramme. Une augmentation de la semi-variance ou une diminution de l'autocorrélation avec la distance est synonyme d'une agrégation (Samalens, 2009).
Un variogramme peut permettre de définir un certain nombre de paramètres ci-dessous (Gosme, 2007):

- L'effet de pépite ⟶ la variance due à une structure spatiale inférieure au grain (le carré de la plus petite distance possible entre points de mesure). L'effet de pépite est représenté par l'ordonnée à l'origine.
- Le seuil → la variance intrinsèque de la variable dans la population. Il est représenté par le niveau asymptotique de la courbe.
- La portée → la distance maximale à laquelle les individus sont corrélés. Elle est représentée par l'abscisse correspondant à la distance où la courbe atteint le seuil.

1.5. Les systèmes de support décisionnel

Les stratégies de lutte contre les agents pathogènes reposent en grande partie sur l'utilisation de fongicides de synthèse, essentiellement à cause de leur faible coût et leur efficacité élevée en absence de résistance. Dans le pathosystème de la pomme de terre, cet engouement pour les fongicides de synthèse n'a pas seulement des impacts négatifs sur l'environnement, il en résulte le développement d'une résistance de *P. infestans* envers ces produits (Fry et Goodwin, 1997). Pour rationaliser l'utilisation de ces produits chimiques, de nombreux systèmes de support décisionnel (SSD) ont été développés partout à travers le monde depuis 1956 (Skelsey, 2008). Ces systèmes (SSD) cherchent à prédire le moment opportun pour l'application des fongicides grâce à une évaluation périodique du risque de développement de la

maladie. La dépendance étroite entre le mildiou de la pomme de terre et les conditions météorologiques a guidé l'élaboration des premiers SSD. Ainsi, les modèles récents de SSD intègrent l'information sur le cycle de vie de l'agent pathogène, les données météorologiques (historiques et prévision), le stade de croissance des plantes, la résistance des cultivars et le type de produits chimiques (temps de dégradation) (Skelsey, 2008a). Une combinaison de l'ensemble de ces paramètres permet d'estimer le risque de développement de la maladie et de recommander des moments de pulvérisation. Cependant, une évaluation menée de 1994 à 1997 dans diverses localités d'Angleterre et du pays de Galles et portant sur cinq SSDs, a révélé que ces systèmes généraient des prévisions différentes et que leurs efficacités variaient d'une année à l'autre. Dans certains cas, le SSD recommandait la pulvérisation de fongicides même si aucune maladie n'avait pas été répertoriée (Day *et al.,* 2001). En effet, la maladie ne se manifeste pas si le pathogène n'est pas présent (Aylor *et al.,* 2010), ce qui peut entraîner une surestimation du risque alors qu'il n'y a pas d'inoculum dans l'air.

1.6. La modélisation et la simulation dynamique

La modélisation d'une épidémie consiste à lui donner une représentation pouvant permettre de décrire et d'analyser son développement (Lepoivre, 2003). Elle représente la hiérarchie et l'enchaînement de divers évènements de base constituant une épidémie. Cependant, elle est une construction de théorique, par ailleurs, la représentation complexe est nécessairement réductionniste et simplificatrice de l'épidémie (Rapilly, 1991). Les modèles peuvent être descriptifs, explicatifs (fonctionnels) et prédictifs (Gosme, 2007).

Les modèles descriptifs sont empiriques, ils ne prennent pas en considération le fonctionnement biologique du pathosystème. Ces modèles ne permettent pas de relier la structure spatiale et le processus épidémiologique, ni de prédire les niveaux de maladie (Carlo et Xavier, 2008).

Les modèles explicatifs ou fonctionnels permettent de faire le lien entre la structure spatiale observée et les processus qui l'ont engendrée pour ainsi tester et générer de nouvelles hypothèses sur le fonctionnement du système (Gosme, 2007).

Les modèles prédictifs permettent de décrire et de prédire la fréquence et la sévérité de l'épidémie. Ils identifient aussi les mesures de contrôle efficaces et fournissent une évaluation des risques de développement de la maladie (Roger *et al.,* 2010). Ils ont pour but de prédire le développement d'une épidémie et la date d'intervention pour le contrôle de la maladie (Rapilly, 1991).

La simulation représente l'information de sortie (quantitative ou qualitative) résultante du modèle (Rapilly, 1991). Les modèles de simulation prédictive sont généralement dynamiques, c'est-à-dire qu'ils prédisent les changements dans une épidémie au fil du temps. Ils permettent de reconstituer étape par étape une épidémie à partir des différentes composantes, par ailleurs, ils nécessitent l'ensemble des informations sur les étapes du cycle épidémiologique de la maladie (Lepoivre, 2003).

L'élaboration et la construction d'un modèle nécessitent qu'un certain nombre de règles soient respectées (Figure 1.13):

- Identifier le pathosystème ⟶ l'objet du modèle doit être identifié (Roger *et al.,* 2010);
- Comprendre l'épidémiologie ⟶ une revue de littérature sur les composantes (agent pathogène, hôte, environnement, etc.) à considèrer et qui rendent dynamique notre système est nécessaire (Rapilly, 1991; Roger *et al.,* 2010);
- Définir les objectifs ⟶ le niveau de compréhension du pathosystème requis va dépendre du degré de prédiction à atteindre (Rapilly, 1991; Roger *et al.,* 2010);
- Élaborer un diagramme schématique du modèle → le diagramme schématique doit comporter les composantes essentielles du modèle, les interactions et indiquer les types d'échanges entre les composantes (Roger *et al.,* 2010);
- Quantifier des relations ⟶ la quantification des relations permet d'établir les relations entre les processus épidémiques sous forme d'équations et d'algorithmes. Elle nécessite souvent qu'on approfondisse l'étape sur la compréhension de l'épidémiologie (Rapilly, 1991; Roger *et al.,* 2010);
- Vérifier et calibrer le modèle → tous les sous-modèles doivent être vérifiés et approuvés avant l'interaction entre les sous-modèles. Cette étape permet de calibrer et de vérifier l'exactitude et la validité des algorithmes (Roger *et al.,* 2010);
- Valider ⟶ ce processus évalue la précision du modèle en comparant les données prédites avec des données observées;
- Vérifier la sensibilité du modèle à un changement de variables d'état en fonction des variables indépendantes est nécessaire. Elle permet de préciser la plage de fonctionnement du modèle, la stabilité des résultats et l'ajustement du modèle (Rapilly, 1991; Roger *et al.,* 2010).
- Identifier les informations de sortie, soit sous forme de graphique, de tableau ou de carte, facilement compréhensible pour les utilisateurs (Rapilly, 1991).

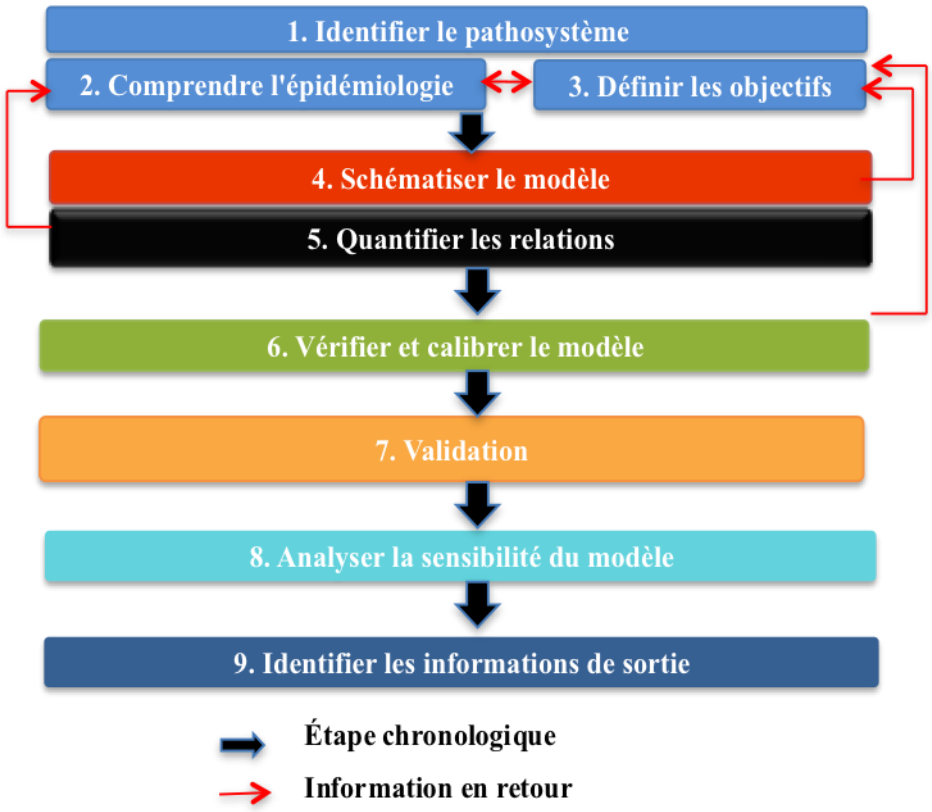


Figure 1.13. Les étapes de développement d'un modèle (Adaptée de Roger *et al.*, 2010; Rapilly, 1991)

1.7. Mise en contexte de la problématique de recherche

Les oomycètes constituent un groupe très diversifié de plus de 500 espèces d'agents pathogènes de plantes, d'animaux, d'insectes, de crustacés, de poissons, etc. (Lamour K., Kamoun, 2009). En agriculture, certains membres de ce groupe sont responsables d'un grand nombre de maladies occasionnant des pertes économiques considérables. Parmi ces maladies dévastatrices en agriculture, on peut citer le mildiou de la pomme de terre et le mildiou de la laitue causés respectivement par

Phytophthora infestans et *Bremia lactucae*. Les pertes économiques mondiales associées au mildiou de la pomme de terre sont estimées entre 3 et 5 milliards de dollars américains par année (Judelson et Blanco, 2005; Haldar et al., 2006). Ainsi, le mildiou de la pomme de terre représente la plus importante menace pour la sécurité alimentaire mondiale selon le centre international de la pomme de terre. Le mildiou de la laitue cause chaque année des pertes importantes de rendement, il est économiquement l'une des plus importantes maladies dans la production mondiale de laitue (Lamour K., Kamoun, 2009).

Le développement de ces deux mildious dépend des conditions climatiques et de la production d'inoculum par les agents pathogènes responsables (Scherm et van Brugger, 1994; Skelsey, 2008d, Fall et al. 2015). L'aérobiologie de *P. infestans* a fait l'objet de plusieurs études dans les 150 dernières années, mais, jamais à l'échelle d'une région de production de monoculture de pomme de terre. Alors que, l'aérobiologie de *B. lactucae* a été peu étudiée dans le monde. Le développement de ces deux mildious durant la saison est lié au cycle de production et dispersion de spores aéroportées. En effet, ce sont des maladies polycycliques à propagation aérienne, ainsi l'inoculum aérien joue un rôle important dans la progression de la maladie. La stratégie mondiale de lutte repose sur l'utilisation de fongicides et de variétés de plantes résistantes (Haldar et al., 2006). La lutte génétique est un des éléments essentiels de la lutte intégrée, mais n'est pas durable puisqu'il y a plusieurs races de *P. infestans* et *B. lactucae* répertoriées (Lamour K., Kamoun, 2009). De plus, de nouvelles races peuvent rapidement émerger suite à l'introduction de nouveaux cultivars (Haldar et al., 2006). Puisqu'il n'y a pas de fongicides biologiques ou d'origines naturelles efficaces, la lutte repose sur l'utilisation de fongicides de synthèse. Ainsi, une lutte durable contre ces mildious passe par l'intégration de méthodes de lutte préventive, génétique, culturale, chimique, une meilleure compréhension de l'aérobiologie des agents pathogènes et la disponibilité d'un outil qui permettrait d'estimer adéquatement les risques de développement de

symptômes. Cependant, la revue de littérature exhaustive sur le mildiou de pomme de terre et de la laitue a permis de mettre en exergue un manque notable de connaissance sur l'aérobiologie des agents pathogènes responsables de ces deux mildious. En conséquence, aucun des modèles de prévision du risque d'apparition des symptômes de ces deux mildious ne prend en considération les facteurs aérobiologiques, telles que, la distribution et l'abondance de l'inoculum aérien, même si, ces derniers représentent les facteurs clefs pour développement du mildiou (Skelsey, 2008d). Pour pallier à ces manquements, trois objectifs ont été fixes.

OBJECTIFS DE LA THÈSE

L'objectif général de ce projet de doctorat était d'améliorer la connaissance sur l'aérobiologie de ces deux oomycètes (*P. infestans* et *B. lactucae*) dans l'optique de mieux comprendre et prédire les périodes d'infection du mildiou de la pomme de terre et de la laitue.

Hypothèse de recherche: Un modèle de simulation dynamique qui intègre l'information sur la quantité d'inoculum et l'efficience contaminatrice pourra permettre de prédire plus efficacement les périodes de risque d'infection du mildiou.

Objectifs spécifiques

Objectif #1. Comprendre la dynamique et la distribution de l'inoculum aérien du mildiou de la pomme terre et du mildiou de la laitue causés respectivement par *Phytophthora infestans* et *de Bremia lactucae*.

Hypothèse #1 : L'inoculum aérien est distribué spatialement de façon hétérogène dans la région étudiée.

Objectif #2. Déterminer le lien entre la concentration de l'inoculum aérien de *P. infestans* et de *B. lactucae* et l'intensité du mildiou (efficience contaminatrice).

Hypothèse #2 : L'intensité du mildiou est quantitativement liée à la concentration de l'inoculum aérien (nombre de sporanges / m^3 d'air).

Objectif #3. Élaborer un modèle de simulation dynamique permettant de prédire les concentrations aériennes de l'inoculum aérien.

Hypothèse #3 : Il est possible de simuler la dynamique de la concentration aérienne de spores.

CHAPITRE 2

VARIATION SPATIO-TEMPORELLE DE LA CONCENTRATION AÉRIENNE DE SPORANGES DE *PHYTOPHTHORA INFESTANS*: UNE CHARACTERISATION ET UNE AVANCÉE VERS L'AMÉLIORATION DE L'ESTIMATION DES RISQUES DE MILDIOU DE LA POMME TERRE

Résumé

L'épidémiologie du mildiou de la pomme de terre, causée par *Phytophthora infestans*, a fait l'objet de plusieurs études dans les 150 dernières années. Les épisodes de mildiou sont fortement influencés par les conditions météorologiques, et cette dépendance constitue l'élément de base pour différents modèles servant à évaluer le moment opportun et la nature des mesures de protection des cultures. Dans la plupart des systèmes de support décisionnels (SSD), les facteurs relatifs à l'inoculum aérien, comme sa présence, sa quantité et sa distribution, sont rarement pris en compte dans l'estimation du risque de mildiou. Cette étude a examiné le potentiel que représente l'utilisation du suivi en temps réel de l'inoculum aérien de *P. infestans* parallèlement aux SSDs. L'expérience a été réalisée pendant les saisons de production de pommes de terre de 2010, 2011 et 2012 dans deux localités du Nouveau-Brunswick, Canada. Les concentrations aériennes de sporanges (CAS) de *P. infestans* ont été mesurées à l'aide 16 capteurs de spores placés à 3 m au-dessus du sol. Les premiers cas de mildiou (2010 et 2011) ont été détectés 6 à 7 jours après l'observation du premier pic de CAS, et tous les capteurs de spores ont effectué leur première capture de sporanges dans la même semaine. Les courbes cumulatives de CAS et les courbes d'estimation de risque de mildiou des deux SSDs (PLANT-Plus et PAMESEB mildiou) avaient les mêmes formes, mais des amplitudes différentes. La

distribution des concentrations aériennes de sporanges s'ajuste mieux à une distribution de la binomiale négative qu'à une distribution de Poisson, ainsi la distribution des sporanges est hétérogène. De même, considérant la loi de puissance de Taylor, l'hétérogénéité augmente avec l'accroissement de la concentration aérienne de spores. Selon l'analyse de covariance, les pentes des modèles de régression de Taylor n'étaient pas significativement différentes entre les années (P = 0,1494, R^2_{adj} = 0,92). Par conséquent, les résultats suggèrent qu'un réseau de capteurs de spores peut être une approche appropriée pour la détection hâtive de l'inoculum provenant de l'extérieur du site de production. De plus, l'utilisation d'un réseau de capteurs en synergie avec un SSD peut aider à une meilleure identification des moments opportuns pour appliquer les produits phytosanitaires. Aussi, les capteurs de spores peuvent être utilisés pour évaluer l'efficacité des stratégies de gestion en utilisant l'aire sous la courbe de progression de l'inoculum. Toutefois, d'autres recherches doivent être menées pour déterminer le nombre optimal de capteurs de spores nécessaires et la relation entre la CAS et l'efficience contaminatrice.

La portée de l'article et les contributions respectives des auteurs

Même si *Phytophthora infestans* a fait l'objet de plusieurs études dans les 150 dernières années, jamais la distribution spatiale de l'inoculum de *P. infestans* n'a été étudiée à l'échelle régionale. Aussi, cette étude a permis, entre autres, de montrer que certains des systèmes de support décisionnels (SSD) utilisés pour rationnaliser l'utilisation des fongicides surestiment et sous-estiment les risques de mildiou de la pomme de terre. Toutefois, l'utilisation d'un réseau de capteurs de spores en parallèle avec les SSDs pourrait permettre d'améliorer l'estimation des risques de mildiou de la pomme de terre.

La contribution des auteurs dans cet article est comme suit : j'ai effectué tous les travaux, les analyses statistiques, la rédaction du manuscrit ainsi que les figures et tableaux pour la publication. M^{r} van der Heyden et D^{r} Carisse ont agi comme conseillers et ont révisé l'article pour sa publication. D^{r} Leclerc, D^{r} Moreau et M^{r} Brodeur ont aidé à la planification stratégique et aux déploiements du réseau de capteurs de spores. Cette recherche a été faite sous la supervision de la D^{r} Carisse.

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SPATIOTEMPORAL VARIATION IN AIRBORNE SPORANGIA OF *PHYTOPHTHORA INFESTANS*: CHARACTERIZATION AND INITIATIVES TOWARD IMPROVING POTATO LATE BLIGHT RISK ESTIMATION

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2.1. Abstract

The epidemiology of potato late blight, which is caused by *Phytophthora infestans*, has been under investigation for the past 150 years. Potato late blight is strongly influenced by weather conditions, and this dependency has been used as a basis for different models to predict whether crop protection measures are required. In most decision support systems (DSS), airborne inoculum parameters, such as presence, amount, and distribution, are rarely considered factors in estimating the risk of late blight. This study investigated the value of using real-time monitoring of *P. infestans* airborne inoculum as a complement to DSS. The experiment was conducted during the 2010, 2011, and 2012 potato production seasons in two locations in New Brunswick, Canada. Airborne sporangia concentrations (ASC) of *P. infestans* were monitored using 16 rotating-arm spore samplers with retracting-type sampling heads placed 3 m above the ground. The first cases of late blight (2010 and 2011) were

detected 6 to 7 d after the first ASC peak, and all samplers captured their first sporangia within the same week (at 3- and 9-d periods). The cumulative ASC curve and the risk curves from two DSS (PLANT-Plus and Pameseb Late Blight) had the same shape but different magnitudes. In both locations, the negative binomial distribution fitted the data better than the Poisson distribution, which is indicative of heterogeneity, and based on Taylor's power law, the heterogeneity increased with increasing ASC. According to analysis of covariance, the slopes of the Taylor's regression models were not significantly different between years ($P = 0.1494$, $R^2_{adj} = 0.92$). Therefore, the present results suggest that spore-sampling network devices may be a suitable approach for early detection of incoming inoculum and, when combined with DSS, represent a potential aid for targeting the optimal time to apply a disease-control product. In this context, cumulative ASC can be a counterweight to the DSS risk estimate: a high risk combined with significant ASC will trigger fungicide spraying. Moreover, spore sampling can be used to assess the efficiency of management strategies by means of examining the area under the inoculum progress curve. Further research needs to be carried out to determine the optimal number of samplers required and the relationship between ASC and infection efficiency.

Keywords: Airborne sporangia, decision support system, spatial variation, spore-sampling network, area under the inoculum progress curve.

2.2. Introduction

Potato late blight, which is caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most destructive potato diseases worldwide (Leonard *et al.*, 2001; Ristaino, 2002). The potential risk of disease development and consequent yield losses

depends in part on the aerial transport of *P. infestans* sporangia to potato fields from off-field sources (Aylor *et al.*, 2011). *Phytophthora infestans* produces asexual sporangia that are adapted to aerial dispersal (Aylor, 2003; Skelsey *et al.*, 2009a). The sporangia may disperse over distances of 40 to 60 km depending on the environmental conditions (Fry & Goodwin, 1997; Aylor *et al.*, 2001). Viable sporangia deposited on the surfaces of susceptible host plants can germinate and invade host tissues or produce zoosporangia. Depending on host susceptibility and environmental conditions, the first symptoms can be visible 3 to 4 days after infection (Cooke *et al.*, 2006). The appearance of symptoms is followed by the production of new sporangia, and the cycle of aerial sporangia dispersal and infection repeats (Skelsey *et al.*, 2008; Aylor *et al.*, 2011). Researchers have documented the crucial role that aerial dispersion of sporangia plays in the development of potato late blight (Skelsey *et al.*, 2009b,c). Dispersal has been recognized as fundamental to the development of plant diseases because, without dispersal, many epidemics may fail to progress (Cooke *et al.*, 2006). Also, knowledge of dispersal processes is needed to understand the movement of pathogens into a landscape (Cooke *et al.*, 2006). In addition to dispersal, the temporal and spatial dynamics of disease epidemics must be understood in order for efficient disease-management systems to be developed (Cooke *et al.*, 2006).

In North America, 576 008 ha of potato were harvested in 2011, with 24% of that production occurring in Canada (Anon., 2012). One of the main potato-producing provinces in Canada is New Brunswick, where potato is the largest cash crop grown and whose 21 651 ha of seeded area borders an area of high potato production in Maine, USA. The province is widely recognized as an international leader in seed potato production and is Canada's largest exporter of seed potatoes. In New Brunswick, despite the availability of decision support systems (DSS), late blight is managed mostly by means of repeated fungicide applications (Y. Leclerc, McCain Foods Ltd., 8800 Main Street, Florenceville, NB, personal communication).

Several DSS for forecasting potato late blight risk have been developed around the world to target fungicide applications to moments when genuine risk is present (Skelsey, 2008). These systems for predicting the risk of infection are considered effective strategies for controlling late blight that make better use of fungicides than fixed-interval fungicide spray programs do (Skelsey, 2008; Aylor *et al.*, 2011). According to the University of California Integrated Pest Management Program (www.ipm.ucdavis.edu), 16 DSS have been developed around the world to determine the optimal time to apply fungicides in order to control late blight. In most DSS, it is assumed that inoculum is present in the area, and control recommendations are driven by the accumulation of periods of favorable weather conditions to predict whether crop protection measures are required. In most DSS, spatial aspects, such as inoculum amount, dispersal, and survival during transportation, are not considered factors in estimating the risk of late blight. These omissions are due to a lack of knowledge about inoculum. *Phytophthora infestans* overwinters in different ways, namely in stored tubers (seed potatoes), in stored infected potatoes that will be discarded in cull piles, and in infected tubers that are not harvested, as well as in volunteer potatoes.

The main difficulty in managing late blight is determining when, where, and how abundant airborne inoculum will be to lead to epidemics. Decision support systems can therefore overestimate the risk when very little or no airborne inoculum is present. This situation needs to be addressed by assessing the risk estimated by a DSS to trigger fungicide intervention in relation to the amount of airborne sporangia. Because late blight is a polycyclic disease with multiple cycles of infection and inoculum production, the infection should increase proportionally to both the initial amount of inoculum present and the amount of new inoculum produced through the course of the epidemic.

To improve strategies for controlling late blight with optimal timing of fungicide applications, it is important to take into account data on the presence of *P. infestans* airborne inoculum in the area as well as the spatial variation in the airborne inoculum. Despite the large amount of scientific information available on late blight, few studies have focused on the value of airborne sporangia concentration (ASC) as a monitoring tool for late blight. Also, there is a lack of information on the spatial distribution of *P. infestans* airborne sporangia at the regional scale as well as over several seasons.

Therefore, the purpose of this study was to examine the value of ASC information as part of a DSS or any other management tool for late blight. Specifically, the study objectives were (*i*) to study the influence of selected weather parameters on ASC and to compare the cumulative disease risk from the two DSS currently used in New Brunswick with cumulative ASC; (ii) to examine the temporal dynamics of *P. infestans* sporangia production; and (iii) to assess the spatial distribution of airborne inoculum of *P. infestans* in the potato production areas of New Brunswick.

2.3. Materials and methods

2.3.1. Data collected

The experiment was conducted in 2010, 2011, and 2012 in two of New Brunswick's potato production areas, representing over 21 000 ha of potato fields. In New Brunswick, potato production is centered on two cities: Florenceville and Grand Falls. Aerial concentrations of *P. infestans* sporangia were measured using a total of 16 rotating-arm spore samplers with retracting-type sampling heads (Phytodata Inc.), 8 in Florenceville and 8 in Grand Falls. Sampler locations were selected based on the

following factors: potential inoculum source, incoming inoculum from long-distance dispersal, and density of potato production. The first factor taken into account was the potential inoculum considering the occurrence of late blight in previous years. The second factor was the vicinity to others potato production areas such as areas of high potato production in Maine, USA. The third factor considered was the density of potato production. The samplers were placed on the edge of each selected field at a height of 3 m above the ground and were operated 3 d per week (Monday, Wednesday, and Friday) from June to September for 4.5 h per sampling period (from 6 a.m. to 3 p.m., alternating between 10 min on and 10 min off). The sampling surfaces of the rods were coated with a thin layer of high-vacuum silicone grease (Fisher Scientific). The effective air sampling rate was 21 L min^{-1}. The sampling efficiency of the rotating-arm samplers for sporangia was estimated in previous studies (rotating-arm sampler efficiency ≈ 0.37 7-d volumetric sampler efficiency) (Noll, 1970; Aylor, 1993). *Phytophthora infestans* sporangia captured on the sampling surfaces (rods) were counted within 24 h after sampling with a microscope at ×400 magnification. The number of sporangia per rod was converted to sporangia per cubic meter as follows: (number of sporangia per rod × 1000 L m^{-3} h^{-1})/(21 L min^{-1} per rod × 60 min h^{-1} × 4.5 h). The locations of each sampler were georeferenced using a global positioning system (Garmin Corp.) and illustrated on a map for the two production areas, Florenceville and Grand Falls (Fig. 2.1).

2.3.2. Meteorological measurements

For each region, air temperature (°C), rainfall and relative humidity (%) were monitored using WatchDog data loggers (Spectrum Technologies, Aurora, IL) located near the spore sampler. Weather data were monitored every 30 min, and hourly averages were used in the analyses. Temperature and RH probes were placed in a

white shelter at 1.5 m above the ground. Rainfall was recorded with a tipping bucket rain gauge (Geneq, Montreal, QC) at a height of 50 cm above the ground.

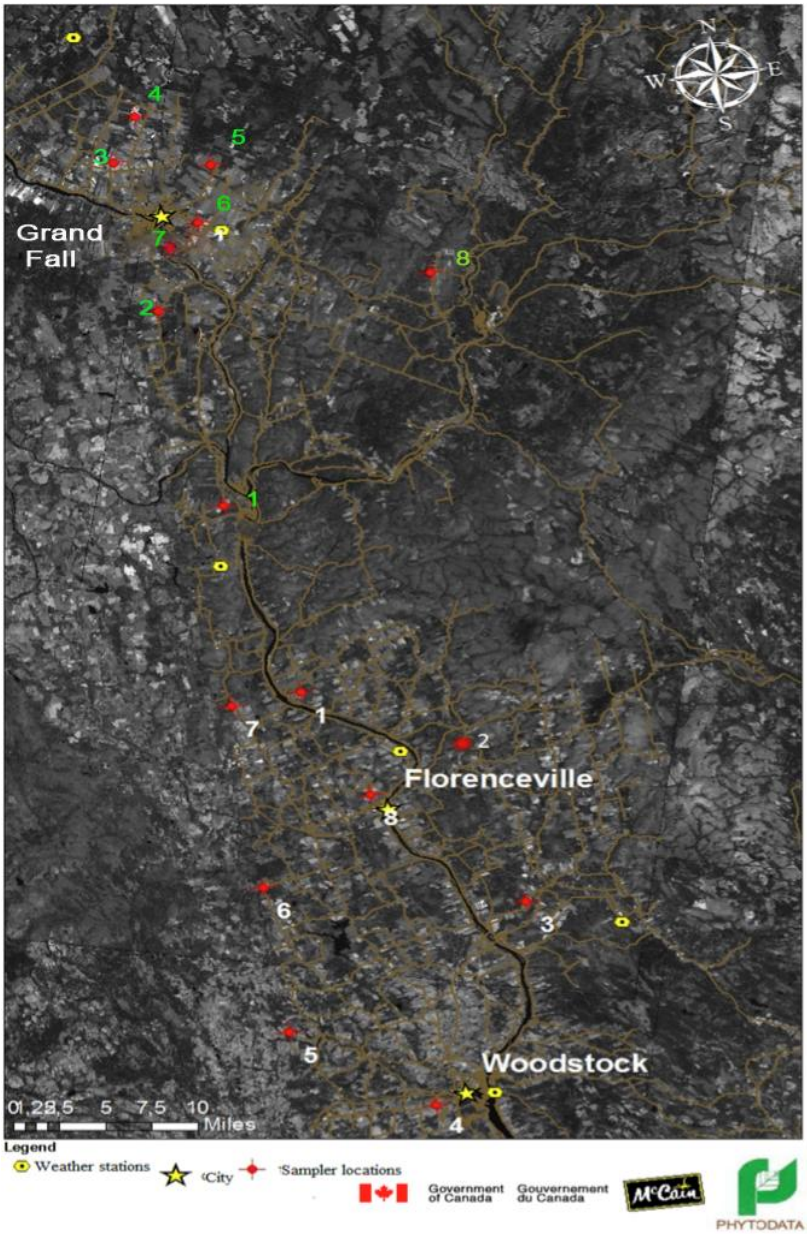

Figure 2.1. Map illustrating the location of spore samplers in New Brunswick, Canada (Florenceville and Grand Falls areas), for the potato production seasons of 2010, 2011, and 2012

2.3.3. Comparative analysis related to Objective (i)

2.3.3.1. Airborne sporangia concentration (ASC) and environmental conditions

Graphical representations of the daily ASC as a function of the number of days after plant emergence were prepared for the Florenceville and Grand Falls sites in 2010, 2011, and 2012. Each graphical representation shows the daily mean ASC (sporangia m^{-3}) detected for each area as well as the environmental conditions (temperature, relative humidity, and rainfall). In New Brunswick, the crop is typically planted in May 1 and emerges 20 to 35 days after planting. Haulm kill occurs from September 5 and 25, with harvest conducted between September 15 and October 10. To evaluate the relationship between ASC and environmental conditions, Kendall's rank correlation analysis was performed. This test provides a coefficient (tau [τ_k]) that quantifies the discrepancy between the number of concordant and discordant pairs. Kendall's tau ranges from −1 to 1, with 0 indicating that there is no correlation between pairs (an α-level of 0.05 is considered to reject the null hypothesis [$\tau_k = 0$]). The data were analyzed using the Kendall function in the Kendall software package (version 2.2, Kendall Rank Correlation and Mann-Kendall Trend Test in Statistical Software R3.0.0 GUI 1.60).

2.3.3.2. Comparison of cumulative airborne sporangia concentration curve and the cumulative risk curves from decision support systems

Graphical representations of cumulative ASC and cumulative risk estimates by DSS as a function of the number of days since June 1 were prepared for 2010, 2011, and 2012. These graphics were prepared for the production areas of Florenceville and Grand Falls considered separately, and eight samplers for each area were taken into account. The two DSS used in the study were PLANT-Plus (Dacom PLANT Service B.V.) and Pameseb Late Blight (Walloon Agricultural Research Center). The accumulated DSS infection risk over the seasons reflects the accumulated number of outbreaks hence the accumulated ASC. Thus, the cumulative infection risk is used in projection of infection events in field. Based on the weather conditions and the life cycle of *P. infestans*, these DSS assess the potential for sporangia germination and penetration and estimate the infection risk in an unprotected crop (using arbitrary units). For Oomycetes (e.g. *P. infestans*), the rate of production of spores (e.g., spores/day) is initially low, then increases and finally decreases. Thus, one way of describing this process is to use the cumulative number of spores (Madden et al. 2007).

2.3.4. Temporal analysis related to Objective (ii): Inoculum progress in time

The four-parameter Weibull model was used to describe cumulative ASC monitored with each sampler as a function of the number of days since plant emergence, as follows:

$$PASC = a\left[1 - \exp\left(-\left(\frac{DAE - lag}{b}\right)^{c}\right)\right]$$

where *PASC* is the proportion of cumulative ASC, *a* is the asymptote and is equal to 1 in this case, *c* is the shape parameter that determines the skewness and kurtosis of the curve, and *b* is the scale parameter regardless of the shape value at the point where *PASC* is 63% of *a*. The *lag* phase refers to the number of days after emergence when the cumulative ASC curve leaves the abscissa (Brown *et al.,* 1988; Eizenberg *et al.,* 2012). Thus, this Weibull equation (with lag phase) allows estimation of the precise time after plant emergence that the samplers captured the first sporangia.

For the analysis of inoculum progress within each sampling unit, the area under the inoculum progress curve (AUIPC) for each sampler was calculated according to the midpoint rule, a method that averages the ASC of two consecutive assessment dates (Madden *et al.*, 2007). The Wilk–Shapiro test was used to assess normality. Analysis of variance and Tukey's multiple comparison tests were performed to detect significant differences between AUIPC for samplers located within the same area and within the same year. The data were analyzed using the analysis of variance, TukeyHSD (honestly significant difference), and pairwise.t.test functions of the Agricolae software package (Statistical Procedures for Agricultural Research, version 1.1-4 in statistical software R3.0.0 GUI 1.60).

2.3.5. Spatial analysis related to Objective (iii)

2.3.5.1. Index of dispersion

The variance-to-mean ratio (V/M), which can be considered a measure of aggregation, was calculated by dividing the sample variance (V) by the sample mean (M). The mean was the arithmetic average of the ASC for each sampling date from June to September. The variance was the square of the deviation of the ASC from the mean. When the V/M is less than 1, equal to 1, or greater than 1, the distribution is considered to be regular, random, or aggregated (heterogeneity), respectively (Madden *et al.*, 2007). Under the null hypothesis of a completely random distribution, $(N - 1) \times$ V/M follows a chi-square distribution with $(N - 1)$ degrees of freedom.

2.3.5.2. Distribution analysis

The heterogeneity of the ASC was evaluated by fitting the Poisson distribution and the negative binomial discrete frequency distribution to the observed ASC data for each year individually (Madden *et al.*, 2007). For each distribution, chi-square goodness-of-fit tests and Akaike's information criterion for parsimony (AIC) were used to determine the best fit. The Poisson distribution has a single parameter, μ, representing the mean of the ASC and is suitable when the number of airborne sporangia is randomly distributed. The negative binomial distribution has two parameters: μ, representing the mean ASC, and k, a measure of the variation (heterogeneity). The negative binomial parameter k is used as an index of dispersion: as k approaches 0, the degree of aggregation increases. The distribution analysis was performed using the SAS software package (version 9.2; SAS Institute Inc.).

2.3.5.3. Taylor's power law

For the ASC data, Taylor's power law (Eqn 3) was used to describe the relationship between the mean (M) and variance (V) of ASC per sampler (Taylor, 1961), as follows:

$$\ln(V) = ln(A) + \beta_1 \ln(M)$$

where ln(A) = β_0 and β_1 are the intercept and the slope of the regression line, respectively.

The significance of the estimated parameters was determined with a *t*-test, and goodness of fit was determined with the coefficient of determination (R^2). A β_1 value greater than 1 is indicative of aggregation (heterogeneity) across all the data sets analyzed. Analysis of covariance was performed to determine the effect of the factor "year" for the data collected in 2010 and 2011. That analysis was conducted to determine if this factor (year) influenced the intercept (β_0) and slope (β_1) parameters of the power law model. The test of a factor is not an indication of whether the factor had a significant effect on ASC, but of whether the factor influenced the ln(V)–ln(M) relationship by affecting the intercept (β_0) and slope (β_1) parameters. The analysis of covariance was performed using the GLM procedure of the SAS software package.

2.4. Results

2.4.1. Airborne sporangia concentration and environmental conditions

The temporal variations in daily ASC of *P. infestans* during the potato production season in Florenceville and Grand Falls in 2010, 2011, and 2012 are shown in Fig. 2.2 In both areas, the highest mean ASC in all the samplers was detected between the end of July and the end of August. In Florenceville, the highest mean ASC was 32.87 sporangia m^{-3} in 2010 (August 9) and 28.71 sporangia m^{-3} in 2011 (August 25) (Fig. 2.2), whereas in Grand Falls, the highest ASC was 61.37 sporangia m^{-3} in 2010 (August 27) and 24.65 sporangia m^{-3} in 2011 (July 29) (Fig. 2.2). In 2012, the ASC in New Brunswick were very low; during that season, most of the daily airborne sporangia counts were zero, except on nine dates, which had very low daily sporangia concentrations (between 0.125 and 2.37 sporangia m^{-3}). Considering that sporangia were trapped in low numbers and on only a few sampling dates in 2012, no analysis was done for these data, except for Kendall's correlation analysis. The concentrations of sporangia in Florenceville and Grand Falls were higher during the 2010 production season than in 2011 and 2012. Agronomists visiting potato fields reported blight outbreaks as follows. In 2010, 2011, and 2012, the first cases of late blight in New Brunswick were detected on July 3, July 20, and July 4, respectively. In 2010 and 2011, the first cases of late blight were discovered 6 to 7 d after the first ASC peak. In 2012, only one case of late blight was reported; this period was followed by a major drought in July and August. However, the outbreaks did not necessarily occurred in fields where samplers were located and confidentiality agreements with growers precluded disclosure of information on disease locations and assessment of its severity. In general, no significant correlation was found between the ASC and the weather conditions (temperature, duration of the period of high humidity, and rainfall) except for a weak significant correlation between the ASC

and the duration of the period of high humidity when considering the combined data from 2010 to 2012 (Table 2.1).

2.4.2. Comparison of cumulative airborne sporangia concentration curve and the cumulative risk curves from decision support systems

Cumulative ASC of *P. infestans* and the cumulative risk estimates by the two DSS (PLANT-Plus and Pameseb Late Blight) in both potato production areas in 2010, 2011, and 2012 are shown in Fig. 2.3. In Florenceville, cumulative ASC reached maxima of 218.40, 133.00, and 10.60 sporangia m^{-3} in 2010, 2011, and 2012, respectively. In contrast, the PLANT-Plus cumulative risk estimates reached maxima of 4325, 7449, and 5231 units of risk 2010, 2011, and 2012, respectively, and the Pameseb Late Blight cumulative risk estimates reached maxima of 4213, 7118, and 5480 units of risk in the same respective years.

In Grand Falls, cumulative ASC reached maxima of 369.44, 122.58, and 7.32 sporangia m^{-3} in 2010, 2011, and 2012, respectively. In contrast, the PLANT-Plus cumulative risk estimates reached maxima of 4543, 10 052, and 6116 units of risk in 2010, 2011, and 2012, respectively, and the Pameseb Late Blight cumulative risk estimates reached maxima of 4680, 4680, and 5480 units of risk in the same respective years. Thus, there is a gap between the magnitude of the risk estimation by DSS and the magnitude of ASC (Fig. 2.3). These results suggest that the two DSS underestimated the late blight risk in 2010 and overestimated it in 2012.

2.4.3. Temporal analysis: Inoculum progress in time

For all samplers, increases in inoculum over the growing season in 2010 and 2011 in both areas were evaluated. Significant differences in the AUIPC among the samplers were found in each year (Table 2.2). In Florenceville, the AUIPC was not significantly different from sampler to sampler in 2010 and 2011 (Table 2.3).

In Grand Falls, the AUIPC measured with sampler 3 was significantly greater than the AUIPC measured with the other samplers in 2010, whereas in 2011, the AUIPC of samplers 3 and 7 were significantly higher than the AUIPC of the other samplers (Table 3.4).

The parameters of the Weibull equation are presented in Tables 2.5 and 2.6. In Florenceville, the lag phases, which estimate the time after plant emergence that the samplers captured the first sporangia, were between 17 and 21 d after plant emergence in 2010 and between 33 and 42 d after plant emergence in 2011. It took 50 to 94 d and 50 to 78 d after plant emergence to achieve 63% of the maximal ASC in 2010 and 2011, respectively (Table 2.5, Fig. 2.4).

Table 2.1. Kendall's correlation coefficients (τ_k) of airborne sporangia concentration (ASC) and weather conditions (air temperature [Tp], relative humidity [RH], and rainfall) in 2010, 2011, and 2012 individually, and in 2010 to 2012 considered together

City	Par	2010		2011		2012		2010–2012	
		τ_k	P	τ_k	P	τ_k	P	τ_k	P
Florence	Tp	0.12	0.37	0.27	0.86	0.14	0.39	0.15	0.08
-ville	RH	0.13	0.27	0.22	0.05	0.02	0.87	0.14	0.02*
	Rainfall	0.08	0.53	−0.06	0.71	0.15	0.06	0.16	0.67
Grand	Tp	0.04	0.61	0.39	0.16	−0.02	0.87	0.09	0.12
Falls	RH	0.03	0.71	-0.01	0.92	0.11	0.39	-0.02	0.71
	Rainfall	0.05	0.64	0.06	0.57	0.11	0.63	0.10	0.13

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively
P-values are for tests to determine whether τ_k is significantly different from 0. Par. for parameter.

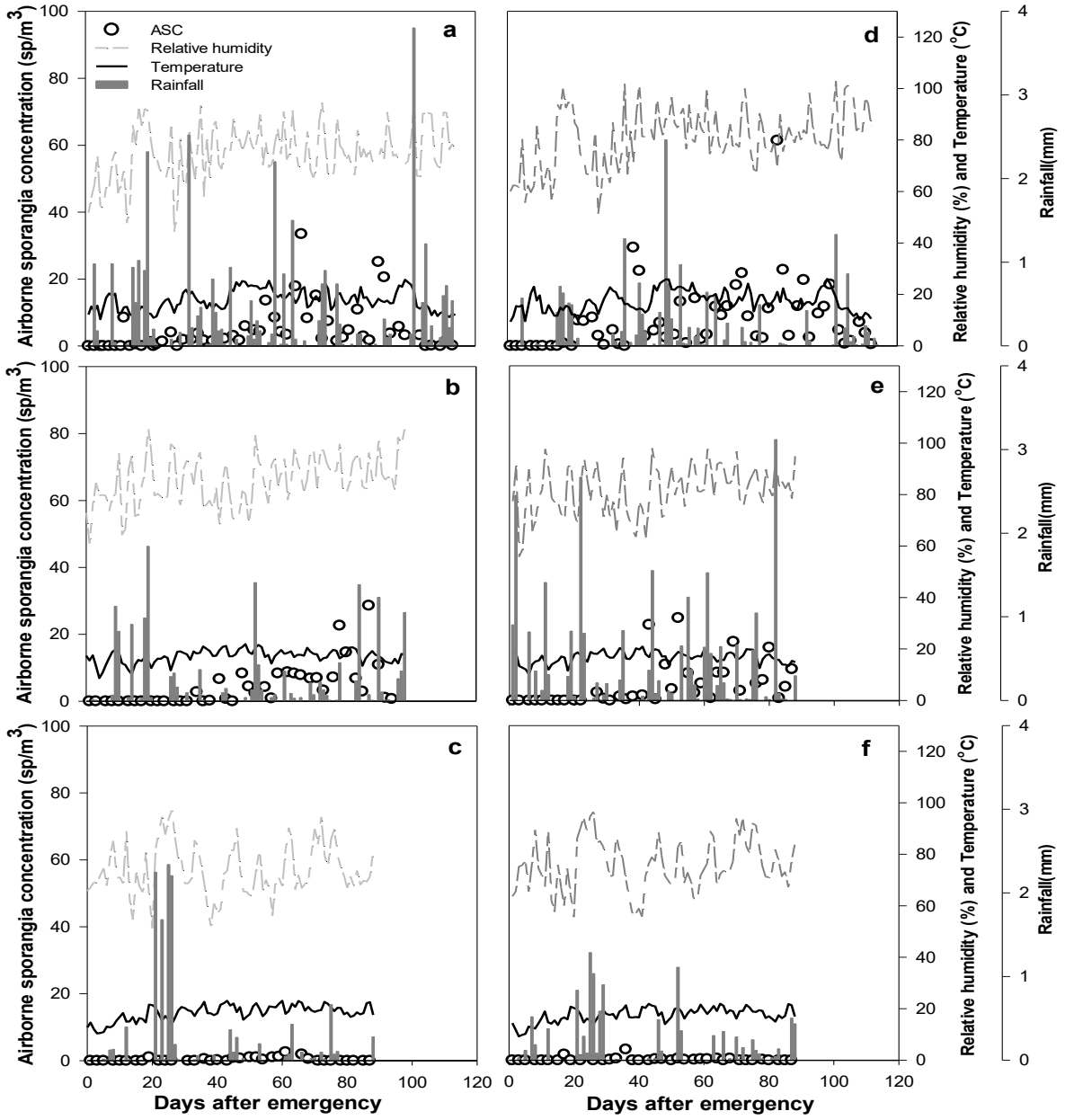

Figure 2.2. Daily airborne sporangia concentrations (ASC; sporangia [sp] m^{-3}) of *Phytophthora infestans* during the potato production seasons in Florenceville in 2010 (a), 2011 (b), and 2012 (c) and in Grand Falls in 2010 (d), 2011 (e), and 2012 (f) in relation to the weather conditions (relative humidity [%], temperature [°C], and rainfall [mm]).

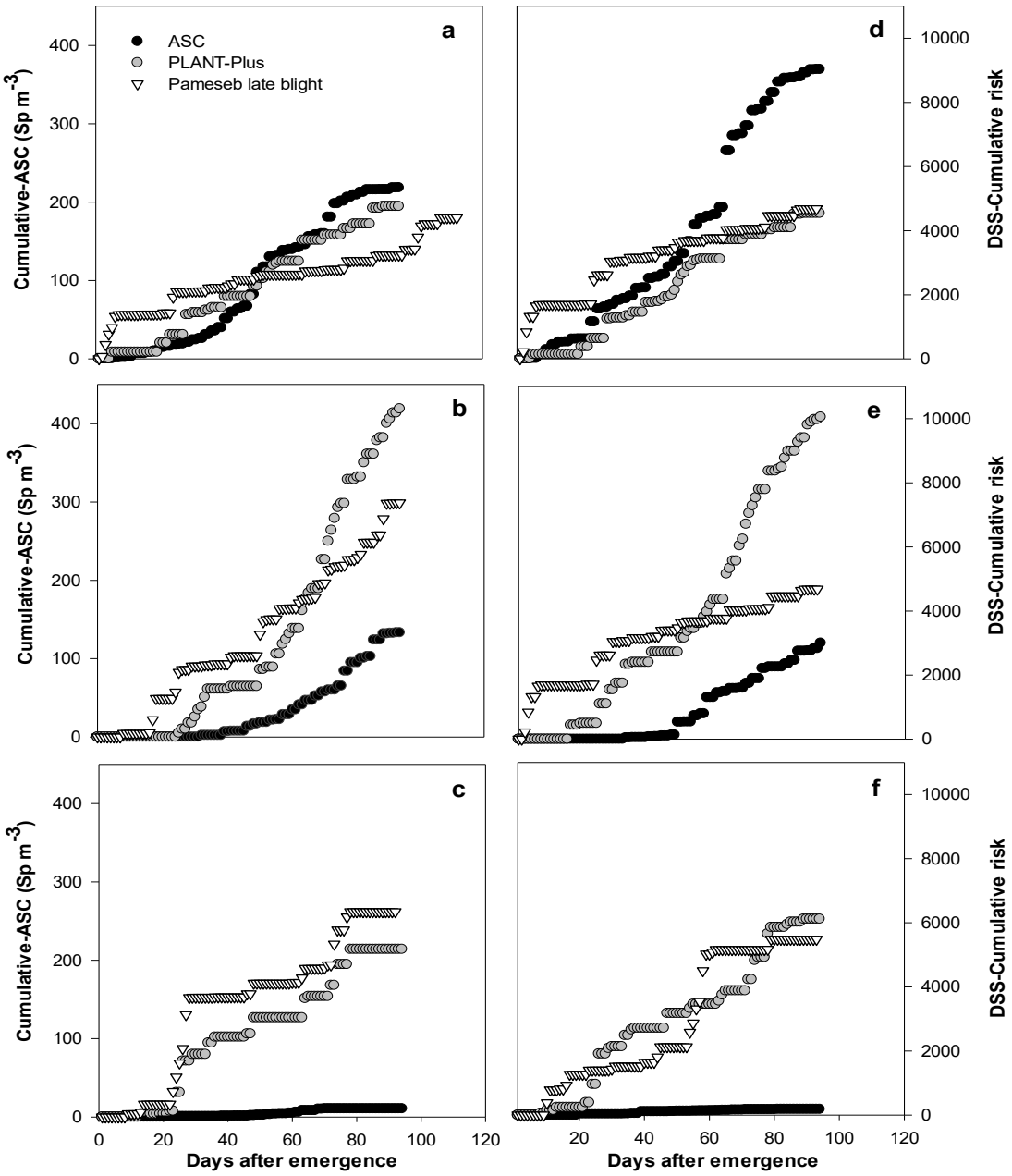

Figure 2. 3. Cumulative airborne sporangia concentrations (ASC; sporangia [sp] m^{-3}) of *Phytophthora infestans* in comparison to the cumulative risk estimates by the decision support systems (DSS; PLANT-Plus and Pameseb late blight) in Florenceville in 2010 (a), 2011 (b), and 2012 (c) and in Grand Falls in 2010 (d), 2011 (e), and 2012 (f).

In Grand Falls, the lag phases were between 11 and 20 d after plant emergence in 2010 and between 22 and 31 d after plant emergence in 2011. It took 72 to 95 d and 41 to 77 d after plant emergence to achieve 63% of the maximal ASC in 2010 and 2011, respectively (Table 2.6, Fig. 2.4). In 2011, samplers 3 and 7 captured their first sporangia 24 and 31 d after plant emergence and reached 63% of the maximal ASC 31 and 36 d after the lag phase (or 55 and 67 d after plant emergence), respectively. Also, those samplers reached 63% of the maximal ASC more rapidly than did the other samplers (except sampler 4). With the exception of samplers 1 and 8 in Florenceville, the number of days to reach 63% of the maximal ASC was greater in 2010 than in 2011.

Table 2.2. Results of analysis of variance on the area under the inoculum progress curves (AUIPC) of cumulative airborne sporangia concentration in Florenceville and Grand Falls in 2010 and 2011

	Florenceville AUIPC				Grand Falls AUIPC			
Year	d.f.[a]	MS[b]	F	*P*[c]	d.f.[a]	MS[b]	F	*P*[c]
2010	6	981005	6.91	6.26e-07 ***	7	507233	21.28	<2e-16 ***
2011	7	7434	2.35	0.0233 *	7	95720	13.8	2.79e-15 ***

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively
[a]Degrees of freedom.
[b]Mean square.
[c]Probability associated with individual

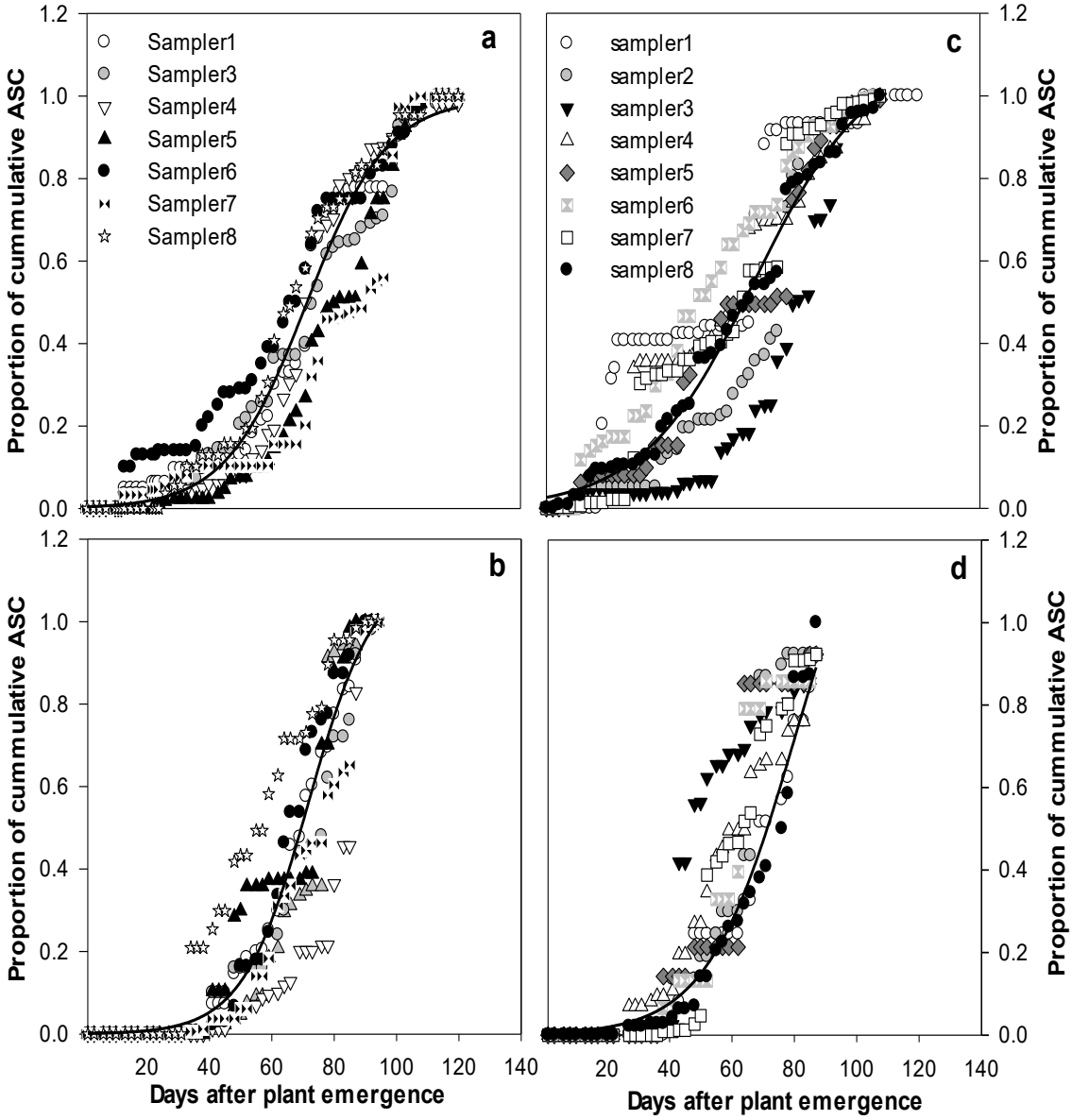

Figure 2.4. Fitted four-parameter Weibull curve of proportion of cumulative airborne sporangia concentration (ASC) as a function of the number of days after plant emergence in Florenceville in 2010 (a) and 2011 (b) and in Grand Falls in 2010 (c) and 2011 (d). The data collected from sampler 2 in 2010 in Florenceville were discarded because of breakdown of the sampling device.

Table 2.3. Results of multiple comparisons of the area under the inoculum progress curves of cumulative airborne sporangia concentration in Florenceville in 2010 and 2011

Florenceville 2010						Florenceville 2011			
Sampler		MD	P_{adj}	95% confidence		MD	P_{adj}	95% confidence	
				LB	UB			LB	UB
S1	S2	-	-	-	-	0.21	1.00	−38.11	38.53
	S3	34.28	1.00	−37.86	106.42	−36.10	0.12	−74.42	2.225
	S4	60.58	0.15	11.55	132.72	−5.93	1.00	−44.26	32.38
	S5	−35.96	0.001***	−108.11	36.17	−26.66	0.76	−64.98	11.66
	S6	−52.46	0.04*	−124.61	19.67	−3.12	1.00	−41.45	35.20
	S7	27.145	0.05*	−44.99	99.28	−4.37	1.00	−42.70	33.95
	S8	−51.78	0.02*	−123.92	20.36	−18.22	1.00	−56.55	20.10
S2	S3	-	-	-	-	−36.31	0.11	−74.63	2.01
	S4	-	-	-	-	−6.15	1.00	−44.47	32.17
	S5	-	-	-	-	−26.87	0.76	−65.20	11.45
	S6	-	-	-	-	−3.33	1.00	−41.66	34.98
	S7	-	-	-	-	−4.58	1.00	−42.91	33.73
	S8	-	-	-	-	−18.43	1.00	−56.76	19.88
S3	S4	26.30	0.15	−45.83	98.44	30.16	0.41	−8.16	68.48
	S5	−70.25	0.05*	−142.39	1.89	9.43	1.00	−28.88	47.76
	S6	−86.75	0.007**	−158.89	−14.60	32.97	0.24	−5.35	71.30
	S7	−7.13	0.05*	−79.27	65.00	31.72	0.30	−6.60	70.05
	S8	−86.06	0.008**	−158.20	−13.92	17.87	1.00	−20.45	56.20
S4	S5	−96.55	0.001***	−168.69	−24.41	−20.72	1.00	−59.05	17.60
	S6	−113.05	0.000***	−185.19	−40.91	2.81	1.00	−35.51	41.13
	S7	−33.43	0.05*	−105.57	38.70	1.56	1.00	−36.76	39.88
	S8	−112.36	0.000***	−184.50	−40.22	−12.28	1.00	−50.61	26.03
S5	S6	−16.50	1.00	−88.64	55.64	23.53	1.00	−14.78	61.86
	S7	63.11	0.12	−9.02	135.25	22.28	1.00	−16.03	60.61
	S8	−15.81	1.00	−87.95	56.32	8.43	1.00	−29.88	46.76
S6	S7	79.61	0.08	7.47	151.75	−1.25	1.00	−39.57	37.07
	S8	0.68	1.00	−71.45	72.82	−15.10	1.00	−53.42	23.22
S7	S8	−78.92	0.09	−151.06	−6.78	−13.85	1.00	−52.17	24.47

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. TukeyHSD function, and the adjusted P-values were obtained using the pairwise.t.test function ($\alpha = 0.05$). (-) The data collected from sampler 2 in 2010 were discarded because of breakdown of the sampling device. MD = mean difference, LB = Lower bound, UB = Upper bound.

Table 2.4. Results of multiple comparisons of the area under the inoculum progress curves of cumulative airborne sporangia concentration in Grand Falls in 2010 and 2011

		Grand Falls 2010				Grand Falls 2011			
Sampler		MD	P_{adj}	95% confidence		MD	P_{adj}	95% confidence	
				LB	UB			LB	UB
S1	S2	11.117	1.00	−85.97	108.20	−3.01	1.00	−63.83	57.80
	S3	78.36	0.20	−18.72	175.45	119.54	1e-07***	58.72	180.35
	S4	128.91	0.001***	31.82	226.00	47.40	0.23	−13.41	108.21
	S5	−28.64	1.00	−125.73	68.44	−8.20	1.00	−69.01	52.61
	S6	27.68	1.00	−69.40	124.76	−7.81	1.00	−68.63	53.00
	S7	298.28	2e-16 ***	201.19	395.37	108.30	2e-06***	47.48	169.11
	S8	84.04	0.13	−13.046	181.13	24.97	1.00	−35.84	85.78
S2	S3	67.24	0.42	−29.84	164.33	122.55	6e-08***	61.74	183.37
	S4	117.79	0.004**	20.70	214.88	50.41	0.16	−10.40	111.23
	S5	−39.76	1.00	−136.85	57.32	−5.18	1.00	−66.00	55.63
	S6	16.56	1.00	−80.52	113.65	−4.80	1.00	−65.61	56.01
	S7	287.17	2e-16 ***	190.08	384.25	111.31	1e-06***	50.49	172.13
	S8	72.92	0.293	−24.16	170.01	27.98	1.00	−32.83	88.80
S3	S4	50.55	1.00	−46.53	147.64	−72.14	0.006**	−13.95	−11.32
	S5	−107.01	0.014*	−204.09	−9.92	−127.74	1e-08***	−188.55	−66.92
	S6	−50.68	1.00	−147.76	46.40	−127.35	1e-08***	−188.17	−66.54
	S7	219.92	5e-10***	122.83	317.01	−11.24	1.00	−72.05	49.57
	S8	5.68	1.00	−91.40	102.76	−94.57	6e-05***	−155.38	−33.75
S4	S5	−157.56	2e-05***	−254.65	−60.47	−55.60	0.08	−116.41	5.21
	S6	101.23	0.025*	−198.32	−4.14	−55.21	0.08	−116.03	5.60
	S7	169.37	4e-06***	72.28	266.46	60.90	0.04*	0.08	121.71
	S8	−44.87	1.00	−141.96	52.21	−22.42	1.00	−83.24	38.38
S5	S6	56.32	0.85	−40.75	153.41	0.38	1.00	−60.43	61.20
	S7	326.93	2e-16***	229.84	424.02	116.50	3e-07***	55.68	177.31
	S8	112.69	0.008**	15.60	209.78	33.17	1.00	−27.64	93.98
S6	S7	270.60	1e-14***	173.51	367.69	116.11	3e-07***	55.29	176.93
	S8	56.36	0.85	−40.72	153.45	32.78	1.00	−28.03	93.60
S7	S8	−214.24	1e-09***	−311.33	−117.15	−83.32	0.000***	−144.14	−22.51

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, MD = mean difference, LB = Lower bound, UB = Upper bound.

2.4.4. Spatial analysis

The V/M ratio was significantly greater than 1 in 96% and 91% of the data sets in New Brunswick in 2010 and 2011, respectively, according to a chi-square test (Fig. 2.5). According to the V/M ratio, heterogeneity of ASC was present at the regional scale for New Brunswick. A comparison of the distribution of observed ASC data with the Poisson distribution and the negative binomial distribution is illustrated in Fig. 2.6.

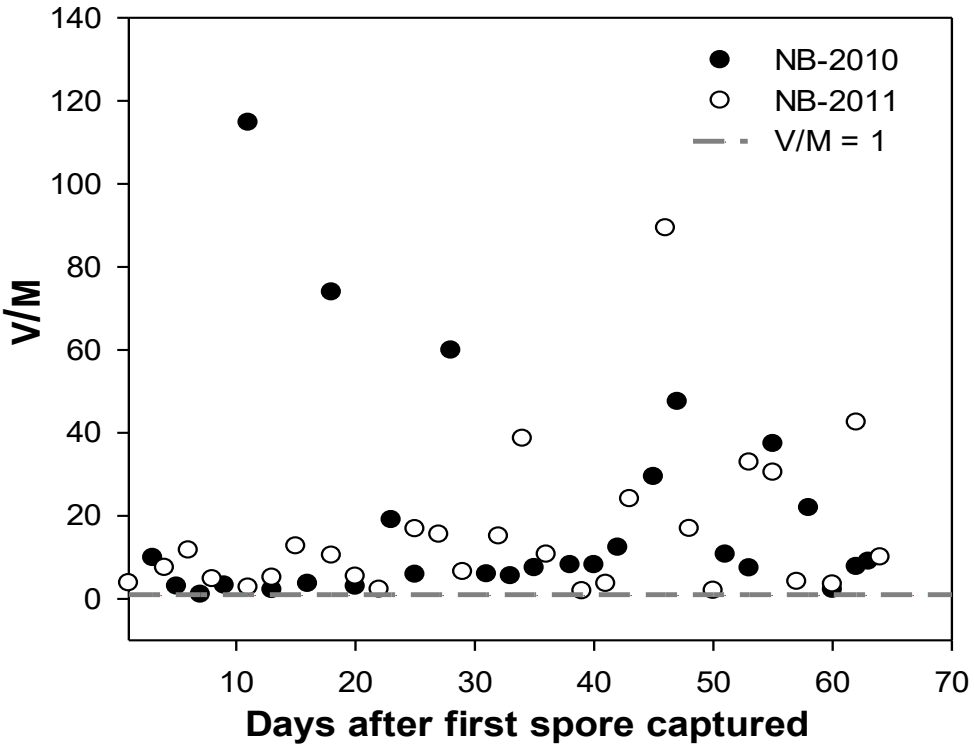

Figure 2.5. Daily variance-to-mean ratio (V/M) of airborne sporangia concentrations (ASC) in the potato production region of New Brunswick in 2010 (NB-2010) and 2011 (NB-2011) as a function of the number of days after the first spore was captured.

Table 2. 5. Estimated parameters for the Weibull equation (Eqn 2) in Florenceville in 2010 and 2011

Florenceville 2010						Florenceville-2011				
Sampler	**R^2_{adj}**	**Parm[a]**	**Estm**	**SE**	**t**	**R^2_{adj}**	**Parm[a]**	**Estm**	**SE**	**t**
		a	1.04	0.03	<2e-16***		a	1.09	0.07	5.30e-16***
S1	0.99	b	51.48	2.94	<2e-16***	0.92	b	78.5	6.65	2.34e-11***
		lag	17.49	1.57	1.23e-14***		lag	32.76	2.36	0.0451*
		a	-	-	-		a	1.05	0.03	8.00e-07***
S2		b	-	-	-	0.98	b	76.4	2.58	6.02e-07***
		lag	-	-	-		lag	33.89	1.34	5.24e-07***
		a	1.29	0.07	<2e-16***		a	1.33	0.09	1.12e-15***
S3	0.98	b	83.29	6.65	<2e-16***	0.96	b	65.4	5.64	1.55e-05***
		lag	17.63	2.36	0.131		lag	40.16	1.70	<2e-16***
		a	1.07	0.03	<2e-16***		a	1.02	0.07	1.30e-07***
S4	0.90	b	49.66	2.58	<2e-16***	0.98	b	49.7	7.87	1.74e-08***
		lag	19.88	1.34	<2e-16***		lag	43.23	2.94	7.21e-10***
		a	1.38	0.09	<2e-16***		a	1.01	0.25	<2e-16***
S5	0.88	b	73.83	5.64	<2e-16***	0.93	b	57.9	12.27	<2e-16***
		lag	19.65	1.70	3.82e-15***		lag	40.3	2.74	1.23e-14***
		a	1.26	0.07	<2e-16***		a	1.21	0.02	<2e-16***
S6	0.99	b	93.87	7.87	1.12e-15***	0.91	b	62.6	2.41	<2e-16***
		lag	17.24	2.94	1.55e-05***		lag	39.5	1.39	<2e-16***
		a	1.60	0.25	1.30e-07***		a	1.03	0.09	1.74e-08***
S7	0.97	b	83.63	12.27	1.74e-08***	0.99	b	58.5	9.84	7.21e-10***
		lag	21.26	2.74	7.21e-10***		lag	42.8	3.71	0.0478*
		a	1.052	0.02	<2e-16***		a	1.02	0.24	1.92e-15***
S8	0.96	b	53.43	2.41	<2e-16***	0.95	b	61.8	16.22	1.05e-05***
		lag	21.61	1.39	1.1e-10***		lag	32.9	3.99	<2e-16***

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

[a]a is the maximal asymptote, b is the number of days after plant emergence at 63% of the maximal asymptote (a), and lag is the number of day after plant emergence until the first detected airborne sporangia concentration. MD = mean difference, LB = Lower bound, UB = Upper bound. (-) The data collected from sampler 2 in 2010 were discarded because of breakdown of the sampling device. Parm =parameter, Estm =estimate, SE = standard error.

Table 2. 6. Estimated parameters for the Weibull equation in Grand Falls in 2010 and 2011

Grand Falls 2010						Grand Falls 2011				
Sampler	R^2_{adj}	Parm[a]	Estm	SE	t	R^2_{adj}	Parm[a]	Estm	SE	T
		A	1.20	0.14	7.91e-11***		a	1.05	0.03	1.92e-15***
S1	0.90	B	94.72	18.61	6.84e-06***	0.90	b	76.9	2.58	1.05e-05***
		lag	17.32	8.30	0.00196**		lag	22.76	1.34	<2e-16***
		A	1.60	0.27	8.00e-07***		a	1.11	0.07	<2e-16***
S2	0.97	B	83.81	13.91	6.02e-07***	0.98	b	66.4	7.87	<2e-16***
		lag	18.99	3.24	5.24e-07***		lag	25.19	2.94	<2e-16***
		A	-	-	-		a	1.32	0.02	1.12e-15***
S3		B	-	-	-	0.95	b	55.7	2.41	1.55e-05***
		lag	-	-	-		lag	24.14	1.39	<2e-16***
		A	1.19	0.09	5.30e-16***		a	1.12	0.07	1.30e-07***
S4	0.99	B	86.73	9.84	2.34e-11***	0.91	b	40.7	7.87	1.74e-08***
		lag	16.77	3.71	0.0451*		lag	23.23	2.94	7.21e-10***
		A	1.56	0.24	9.96e-08***		a	1.00	0.09	1.30e-07***
S5	0.98	B	100.76	16.22	1.51e-07***	0.94	b	67.7	9.84	1.74e-08***
		lag	19.71	3.99	0.624		lag	29.3	3.71	7.21e-10***
		A	1.11	0.02	<2e-16***		a	1.01	0.02	<2e-16***
S6	0.96	B	72.38	3.03	<2e-16***	0.91	b	52.8	2.41	<2e-16***
		lag	15.63	1.48	0.00045***		lag	29.5	1.39	<2e-16***
		A	1.26	0.12	2.66e-13***		a	1.31	1.26	1.74e-08***
S7	0.99	B	80.73	11.07	3.75e-09***	0.99	b	68.7	80.73	7.21e-10***
		lag	18.08	4.19	0.797		lag	31.8	18.08	0.0478*
		A	1.41	0.11	3.88e-16***		a	1.34	0.24	1.92e-15***
S8	0.98	B	94.03	8.45	1.69e-14***	0.97	b	51.9	16.22	1.05e-05***
		lag	11.62	2.40	0.0503*		lag	22.9	3.99	<2e-16***

Signif. codes: *, **, and *** represent $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

[a]a is the maximal asymptote, b is the number of days after plant emergence at 63% of the maximal asymptote (a), and lag is the number of day after plant emergence until the first detected airborne sporangia concentration.

(-) The Weibull (Eq2) did not fit the data. Parm =parameter, Estm =estimate, SE = standard error.

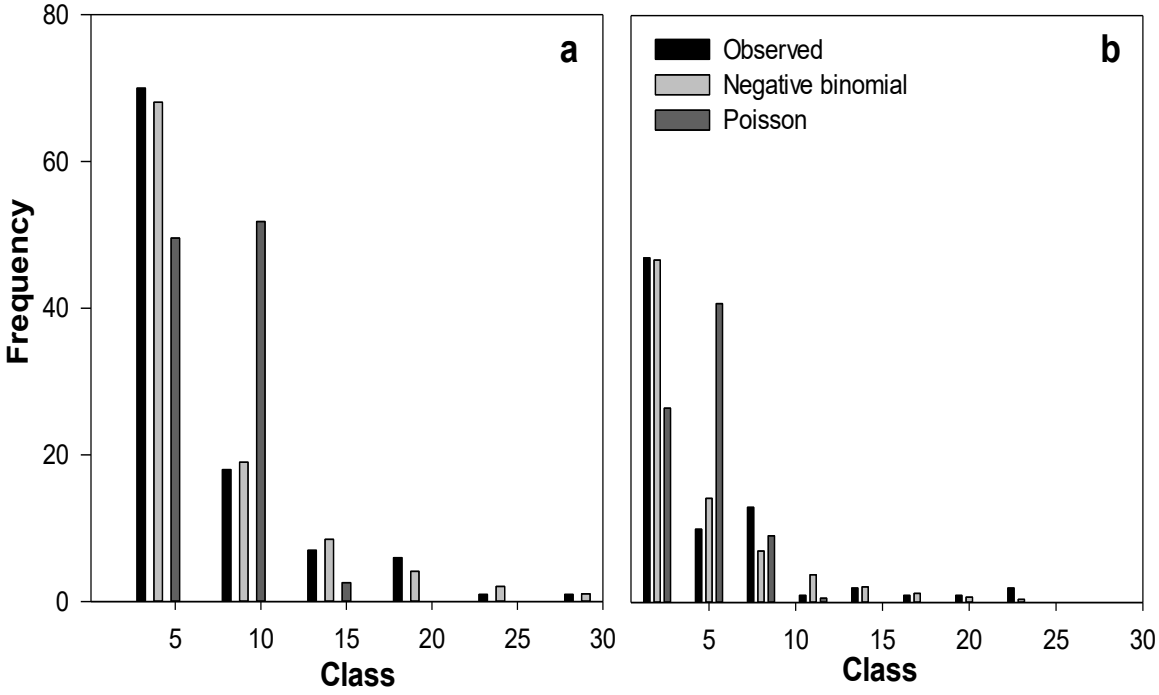

Figure 2.6. Frequency distributions representing the means of airborne sporangia per sampling day in New Brunswick in 2010 (a) and 2011 (b). The black bars represent observed frequencies, the light gray bars represent expected negative binomial frequencies, and the dark gray bars represent expected Poisson frequencies.

The negative binomial distribution provided a better fit to the data than did the Poisson distribution (Fig. 2.6), according to a chi-square goodness-of-fit test and the AIC. The negative binomial distribution fit 74.12% of the data sets in 2010, and the value of the index of dispersion, *k*, was between 0.16 and 3.3, whereas in 2011, the negative binomial distribution fit 90.6% of the data sets, with a *k* value between 0.13 and 3.5 (Fig. 2.6). In 2011, there was an excess of values in the tails (class < 2 or class > 10; Fig. 2.6) of the observed distribution and a deficit near the mean, in

comparison with the expected Poisson frequencies. These results suggest that the distribution was heterogeneous.

Taylor's power-law graphs, describing the relationship between the observed variance and the mean ASC, are presented in Fig. 2.7. The variance in ASC was proportional to the power of the mean. Taylor's power law provided a good fit to the observed ASC data in New Brunswick (R^2 = 0.95 in 2010; R^2 = 0.94 in 2011). For both years, the estimated slope and intercept parameters were greater than 1 and 0, respectively. In addition, there was heterogeneity (aggregation) across all the data sets analyzed, and that heterogeneity increased with increasing ASC. The analyses of covariance showed that the year did not significantly influence the interaction between the slope and year (F = 2.10, P = 0.1494, R^2_{adj} = 0.92). The spatial pattern provide information about the epidemic spread because epidemics resulting from spatially homogeneous initial spore loads, responded more strongly to the size of the initial spore load than focal epidemics (Skelsey *et al.*, 2009). Also, if spore loads are homogeneous, one sampler is sufficient to measure the ASC at the regional scale.

2.5. Discussion

Various types of DSS have been developed around the world to manage late blight of potato. An earlier evaluation of some of these systems (Taylor et al.,2003) showed that their effectiveness varied from year to year and that they may recommend spraying even when no blight is present. One reason for the lack of accuracy of DSS is that they do not take into account the presence or absence of inoculum in the air, even though it has been documented that inoculum dispersal information is a key factor in late blight epidemic development (Aylor *et al.*, 2001; Skelsey *et al.*, 2009c).

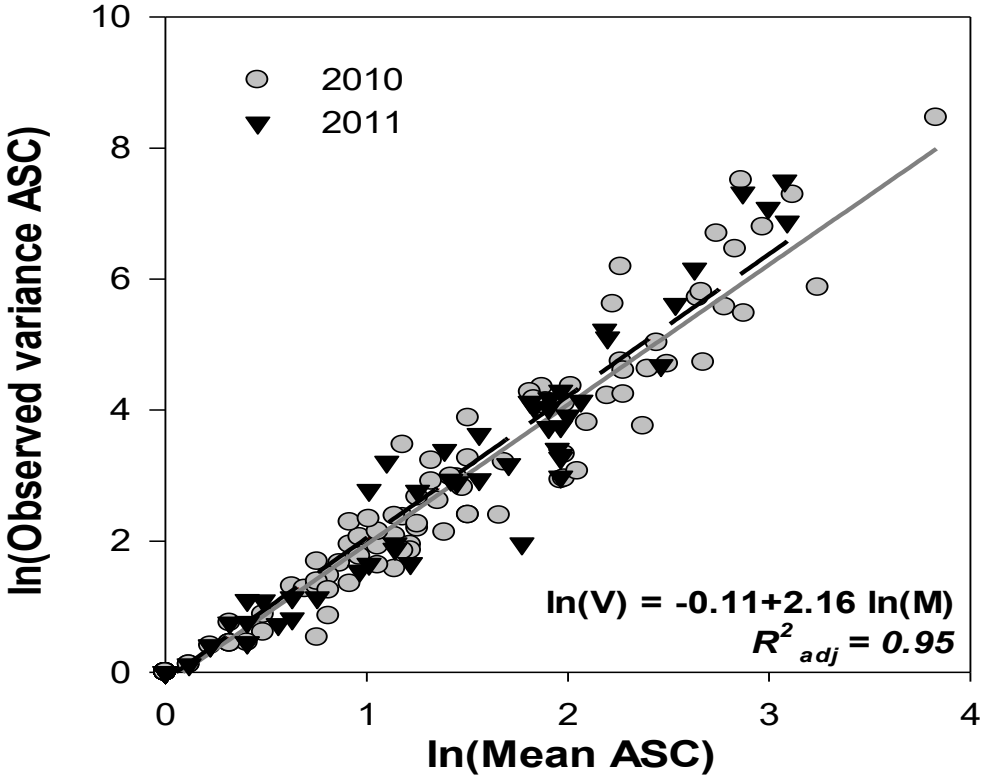

Figure 2.7. Relationship between the logarithm of the observed variance, ln(V), and the logarithm of the mean airborne sporangia concentration (ASC), ln(M), for *Phytophthora infestans* at the regional scale for the New Brunswick potato production area in 2010 and 2011.

Therefore, the main question is how ASC information (inoculum amount, spatiotemporal variation in inoculum) can be integrated into late blight management systems. A key message in the present paper is the fact that in addition to a DSS, a sustainable late blight management system requires quantification of the inoculum amount and a better understanding of the spatiotemporal variation in the inoculum

(sporangia). To date, few research efforts have focused on the spatiotemporal variation of *P. infestans* sporangia above potato fields. The present study sought to assess whether ASC information can add value to DSS and improve the decision-making process for the management of potato late blight. Therefore, in this study, the influence of temperature, relative humidity, and rainfall on ASC was assessed, and cumulative ASC values were compared with cumulative disease risk. The temporal and spatial dynamics of *P. infestans* sporangia were studied in two large, uniform potato production areas (New Brunswick, Canada) encompassing more than 21 ha by means of 16 samplers (8 in Florenceville and 8 in Grand Falls) at a height 3 m above the ground.

The daily mean counts of *P. infestans* ASC revealed significant variations in ASC over the course of each sampling period in 2010 and 2011. The first cases of late blight in New Brunswick (2010 and 2011) were detected between 6 and 7 d after the first ASC peak (Fig. 2.2). These results are supported by the findings of Bugiani *et al.* (1995) for tomato late blight, which indicated an increase in ASC during the early weeks before the first disease symptoms were detected in the field. In Florenceville, all samplers captured the first sporangia within a 3- and 9-d period in 2010 and 2011, respectively, whereas in Grand Falls, the first sporangia were captured within a 9-d period in 2010 and 2011 (Tables 2.5 and 2.6, Fig. 2.4). Also, there was a weak correlation between the ASC and selected weather parameters (Table 2.1). These results differ from those reported by Iglesias *et al.* (2010), who found a significant correlation between ASC of *P. infestans* and meteorological factors. Therefore, it is reasonable to suggest the hypothesis that early in the season, most sporangia captured at a height of 3 m were attributable to long-distance transport (incoming sporangia or off-farm inoculum sources). However, as the season progressed, the sporangia that were captured probably consisted of a mixture of in-field inoculum sources and off-farm sources. Consequently, a spore-sampling network may be a

suitable approach for early detection of incoming inoculum and can be used in parallel with a DSS for improved within-season disease risk estimation.

The cumulative risk estimates by the DSS were greater in 2011 and 2012 than in 2010, whereas the cumulative ASC and the late blight epidemic were greater in 2010. In 2012, the risk estimates by the DSS were high in comparison with those for the previous two years, even though only one case of late blight was reported in 2012; as well, the ASC was low in that year. These results suggest that the two DSS underestimated the late blight risk in 2010 and overestimated it in 2012 (Fig. 2.3). Decision support systems estimate the potential for the disease to occur and cannot measure the real inoculum level. In this context, cumulative ASC can be a counterweight to the risk estimation by a DSS: a high risk combined with significant ASC will trigger fungicide spraying. Thus, the main question is what ASC level should be considered significant. Contrary to folk wisdom, low sporangia loads do not lead to appreciable crop loss (Skelsey *et al.*, 2009c), and thus further studies should be done to determine the action threshold. The results of the present study suggest that spore-sampling network devices coupled with DSS are useful for targeting the optimal time to apply a disease-control product. Thus, farmers in New Brunswick have started using this kind of spore-sampling network as a functional strategy to control late blight epidemics (Y. Leclerc, McCain Foods Ltd., Florenceville, NB, personal communication). In Michigan, field advisers monitor airborne *Venturia inaequalis* ascospores in apple orchards with rotating-arm samplers and use automated information lines, fax networks, and e-mail to keep producers informed (Cooke *et al.*, 2006). Moreover, spore sampling has the potential to be used to assess the efficiency of management strategies by means of examining the AUIPC.

Significant differences in AUIPC among the samplers were found in 2010 and 2011. In Florenceville, the AUIPC were not significantly different from sampler to

sampler (Table 2.3). Thus, it can be assumed that disease management was uniform and the progression of the epidemic was similar in this production area. In Grand Falls, the AUIPC of sampler 7 in 2010 was significantly higher than the AUIPC of the other samplers, whereas in 2011, the AUIPC of samplers 3 and 7 were higher than the AUIPC of the other samplers (Table 2.4). Also, considering the lag phase before detection of the first sporangia, samplers 3 and 7 reached 63% of the maximal ASC more rapidly than did the other samplers. Therefore, it can be concluded that there was inefficient disease management in the fields covered by sampler 3 in 2010 and by samplers 3 and 7 in 2011. To improve the reliability of the information derived from the spore-sampling network, it is imperative to know whether the number of samplers is sufficient to obtain representative ASC for the study area, a question to which spatial analysis can provide a preliminary response.

Based on the V/M and Taylor's power law, the ASC was heterogeneously distributed during the production seasons in 2010 and 2011 in both Florenceville and in Grand Falls. Also, according to the AIC, there was a better fit to the negative binomial distribution than to the Poisson distribution for all data sets from each area and each year. Based on Taylor's power law, the results suggest that increasing ASC influenced heterogeneity. According to analysis of covariance, the slopes of Taylor's regressions did not differ significantly between years (2010 and 2011) in New Brunswick ($P = 0.1494$, $R^2_{adj} = 0.92$). These results show that the distribution of ASC was heterogeneous in both years and that the more the number of sporangia increased, the greater the heterogeneity became. Also, a spore-sampling network requires several samplers in order to be representative of the study area and to provide more accuracy. This dispersal information can be used to guide decision making. General epidemics, resulting from spatially homogeneous initial sporangia loads, were found to respond more strongly to the size of the initial sporangia load (Skelsey *et al.*, 2009c). Thus, epidemics of late blight respond less to the size of the

initial sporangia load and require monitoring of the inoculum throughout the production season.

The present study examined the potential added value of ASC information in management strategies for potato late blight. The results showed that a spore-sampling network can be complementary to a DSS. Additionally, a network provides day-to-day information about disease progress and the efficiency of management strategies. Also, advances in molecular biology will allow rapid sporangia counts and the identification of pathotypes, thus providing crucial information about the pathogen's virulence and sensitivity to fungicides.

To the present authors' knowledge, this study is the first time that the area under the inoculum progress curve has been proposed as a potential method to assess disease progression and as a monitoring tool for potato late blight. This study lays the groundwork for the potential practical use of the area under inoculum progress curves. In New Brunswick, most potato growers have signed an agreement with McCain Foods Ltd. and must manage their field according to a specific guideline. Hence, it is possible to envision that the spore-sampling network can be implemented at a regional scale and the cost of network installation and management as well as ASC information could be shared among growers. An information dissemination structure is already in place. Also, a similar network was successfully implemented in organic soil in southwest of Montreal to manage leaf blight of onion. Nevertheless, further research should be done to determine the number of samplers required depending on the size of the production area and to identify the link between the number of captured sporangia and infection efficiency. Such information can be used to improve disease support systems by integrating day-to-day information on the presence or absence of sporangia.

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2.7. References

Aylor DE, 1993. Relative collection efficiency of Rotorod and Burkard spore samplers for airborne *Venturia inaequalis* ascospores. *Phytopathology* **83**, 1116–9.

Aylor DE, 2003. Spread of plant disease on a continental scale: Role of aerial dispersal of pathogens. Ecology 84, 1989–97.

Aylor DE, Fry WE, Mayton H, Andrade-Piedra JL, 2001. Quantifying the rate of release and escape of Phytophthora infestans sporangia from a potato canopy. Phytopathology 91, 1189–96.

Aylor DE, Schmale III DG, Shields EJ, Newcomb M, Nappo CJ, 2011. Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agricultural and Forest Meteorology 151, 251–60.

Anonymous, 2012. Canadian potato production. Statistic Canada, service bulletin Catalogue no. 22-008-X 10, 1-14.

Bugiani R, Govoni P, Bottazzi R, Giannico P, Montini B, Pozza M, 1995. Monitoring airborne concentration of sporangia of Phytophthora infestans in relation to tomato late blight in Emilia Romagna, Italy. Aerobiologia 11, 41–46.

Brown, R.F., Mayer, D.G., 1988. Representing cumulative germination. The use of the Weibull function and other empirically derived curves. Ann. Bot. 61, 127–138.

Cooke BM, Jones DG, Kaye B, eds, 2006. The Epidemiology of Plant Diseases, second ed. Dordrecht, The Netherlands: Springer, 576 pp.

Eizenberg, H, Hershernhorn, J, Achdari G., Ephrath, J., E (2012). A thermal time model for predicting parasitism of Orobanche cumana in irrigated sunflower—Field validation. Field Crops Research 137, 49–55.

Fry WE, Goodwin SB, 1997. Resurgence of the Irish potato famine fungus. BioScience 47, 363–71.

Fry, W. E., McGrath, M. T., Seaman, A., Zitter, T. A., McLeod, A., Danies, G., Small, I. M, Myers, K., Everts, K., Gevens, A. J., Gugino, B.K., Johnson, S. B., Judelson, H., Ristaino, J., Roberts, P., Secor, G., Seebold, K., Snover-Clift, K., Wyenandt, A., Grunwald, N. J., and Smart, C. D. (2013). The 2009 late blight pandemic in Eastern USA-causes and results. Plant disease 97 296-306.

Iglesias I, Escuredo O, Seijo C, Méndez J, 2010. Phytophthora infestans prediction for a potato crop. America Journal of Potato Research 87, 32–40.

Leonard R, Dowley LJ, Rice B, Ward S, 2001. Comparison of the NegFry decision support system with routine fungicide application for the control of potato late blight in Ireland. Potato Research 44, 327–36.

Madden LV, Hughes G, van den Bosch F, 2007. The Study of Plant Disease Epidemics. St. Paul, MN, USA: The American Phytopathological Society, 421 pp.

Noll KE, 1970. A rotary inertial impactor for sampling giant particles in the atmosphere. Atmospheric Environment 4, 9–19.

Ristaino JB, 2002. Tracking historic migrations of the Irish potato famine pathogen, Phytophthora infestans. Microbes and Infection 4, 1369–77.

Skelsey P, 2008. Multi-Scale Modeling of Potato Late Blight Epidemics. Wageningen, The Netherlands: Wageningen University, PhD thesis, 257 pp.

Skelsey P, Holtslag AAM, van der Werf W, 2008. Development and validation of quasi-Gaussian plume model for transport of botanical spores. Agricultural and Forest Meteorology 148, 1383–94.

Skelsey P, Kessel GJT, Holtslag AAM, Moene AF, van der Werf W, 2009a. Regional spore dispersal as a factor in disease risk warnings for potato late blight: A proof of concept. Agricultural and Forest Meteorology 149, 419–30.

Skelsey P, Kessel GJT, Rossing WAH, van der Werf W, 2009b. Parameterization and evaluation of a spatiotemporal model of potato late blight pathosystem. Phytopathology 99, 290–300.

Skelsey P, Rossing WAH, Kessel GJT, van der Werf W, 2009c. Scenario approach for assessing the utility of dispersal information in decision support for aerially spread plant pathogens, applied to Phytophthora infestans. Phytopathology 99, 887–95.

Taylor LR, 1961. Aggregation, variance and the mean. Nature 189, 732–5.

Taylor MC, Hardwick NV, Bradshaw NJ, Hall AM, 2003. Relative performance of five forecasting schemes for potato late blight (Phytophthora infestans) I. Accuracy of infection warnings and reduction of unnecessary, theoretical, fungicide applications. Crop Protection 22, 275–283.

CHAPITRE 3

L'EFFICIENCE CONTAMINATRICE DE *BREMIA LACTUCAE* DANS LA CULTURE DE LAITUE AU QUÈBEC EST MODULÉE PAR LA TEMPÉRATURE ET LA DURÉE D'HUMECTATION DU FEUILLAGE

Résumé

Plus de 80% de la production canadienne de laitue est située dans la province du Québec. Toutefois, une grande partie de nos connaissances sur l'épidémiologie du mildiou de la laitue (*Bremia lactucae*) provient d'études réalisées en condition contrôlée ou d'études de terrain menées dans des climats subtropicaux. Par ailleurs, ces connaissances ne peuvent pas être directement transposables à la production de laitue du Québec. L'influence de la température et la durée de mouillure des feuilles sur l'efficience contaminatrice (IE) de *B. lactucae* a été étudiée dans des conditions de champs et en chambre de croissance. L'IE a été définie comme le rapport entre le nombre de lésions par feuilles et la concentration aérienne de conidies (CAC). La CAC de *B. lactucae* a été mesurée trois fois par semaine à l'aide de capteurs de spores. De plus, durant la période de fonctionnement des capteurs de spores, 72 plants indicateurs ont été exposés dans des champs de laitue. L'intensité du mildiou a été évaluée après 7 jours d'incubation des plants indicateurs. En condition contrôlée, une CAC d'une conidie / m^3 était suffisante pour provoquer une lésion par feuille, et l'IE variait entre 0,25 et 1,00. Dans le cas de conditions non contrôlées, une CAC de 10 à 14 conidies / m^3 a été nécessaire pour provoquer une lésion par feuille, et l'IE variait entre 0,02 et 0,10, sauf en 2004 où l'IE variait entre 0,03 et 1,00. L'IE augmente avec la durée d'humectation mais diminue avec la température. En outre, à l'intérieur d'une plage des températures moyennes observées de 10 à 20 °C, une

durée 2 h de mouillure du feuillage était suffisante à *B. lactucae* pour établir une infection. Par conséquent, sous les conditions climatiques du Québec, une période d'humectation de 2 h et une CAC de 10 à 14 conidies / m^3 peuvent être utilisées comme indicateurs de risque pour faciliter la prise de décisions dans la gestion du mildiou. Aussi, sous les conditions météorologiques du Québec, les mesures de durée de mouillure du feuillage en matinée et en soirée devront être utilisées pour améliorer la fiabilité de la durée d'humectation comme indicateur de risque de mildiou. Des recherches complémentaires sont nécessaires pour valider ces indicateurs de risque dans l'optique de les intégrer dans les stratégies de gestion du mildiou de la laitue.

La portée de l'article et les contributions respectives des auteurs

Cette étude a permis entre autres d'établir pour une première la fois la relation entre la concentration aérienne de spores de *Bremia lactucae* et l'intensité du mildiou de la laitue pour ainsi définir un seuil qui pourra être utilisé pour rationaliser l'usage des fongicides. Cet article a permis de voir que sous nos conditions climatiques (Québec), on ne peut pas transposer littéralement les études réalisées dans les régions subtropicales. En effet, cette étude démontre que *Bremia lactucae* a besoin de 2h de mouillure du feuillage pour établir une lésion contrairement aux 3 à 4h mentionnées dans les études réalisées en région subtropicale.

La contribution des auteurs dans cet article est comme suit : j'ai effectué tous les travaux, les analyses statistiques, la rédaction du manuscrit ainsi que les figures et tableaux pour la publication. M^r van der Heyden et D^r Carisse ont agi comme conseillers et ont révisé l'article pour sa publication. Cette recherche a été sous la supervision de D^r Carisse et de D^r Beaulieu.

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BREMIA LACTUCAE INFECTION EFFICIENCY IN LETTUCE IS MODULATED BY TEMPERATURE AND LEAF WETNESS DURATION UNDER QUEBEC FIELD CONDITIONS

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3.1. Abstract

More than 80% of Canadian lettuce production is located in the province of Quebec. Yet most of our knowledge on the epidemiology of lettuce downy mildew (*Bremia lactucae*) is derived from controlled-condition experiments or field experiments conducted in subtropical climates and, thus, cannot readily be applied to Quebec lettuce production. The influence of temperature and leaf wetness duration on the infection efficiency (IE) of *B. lactucae* was studied for 4 years (2003, 2004, 2012 and 2013) under field and growth-chamber conditions. IE was defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (ACC). *B. lactucae* ACC was measured with rotating-arm samplers three times/week. In addition, 72 lettuce trap plants/sampling day were exposed to the potential airborne *B. lactucae* inoculum and disease intensity was assessed after 7 days of incubation of

the trap plants in greenhouse. Under growth chamber conditions, an ACC of 1 conidium/m^3 was sufficient to cause 1 lesion/leaf, and IE ranged from 0.25 to 1.00. Under field conditions, an ACC of 10 to 14 conidia/m^3 was required to cause 1 lesion/leaf, and IE ranged from 0.02 to 0.10, except in 2004 when IE ranged from 0.03 to 1.00. IE increased with increasing leaf wetness duration but decreased with increasing temperature. Also, considering an observed average temperature range from 10 to 20°C in the area of Quebec, 2 h of leaf wetness was sufficient for infection by *B. lactucae*. Therefore, under Quebec lettuce production conditions, a leaf wetness period of 2 h and an ACC of 10 to 14 conidia/m^3 can be used as risk indicators to facilitate disease management decisions. Also, under typical Quebec weather conditions, measuring both morning and evening leaf wetness events could be used to improve the reliability of leaf wetness duration as a downy mildew risk indicator. Further research is needed to validate these risk indicators for integration into management strategies.

3.2. Introduction

Lettuce (*Lactuca sativa*) downy mildew, caused by the oomycete *Bremia lactucae* Regel, is a major threat in lettuce production worldwide (13). Spores of *B. lactucae* have lost the ability to form zoospores and germinate directly on a leaf surface (11). Thus, the term “conidia” is used in this study instead of “sporangia”, although the latter term is used commonly in the literature (7,21).

Despite the presence of the disease every year, outbreaks of lettuce downy mildew are typically sporadic and associated with specific environmental conditions (13). The potential risk of disease development and consequent yield losses also depend on the amount of *B. lactucae* conidia in lettuce fields. *B. lactucae* produces conidia that

are adapted to aerial dispersal (6,26). They are produced when relative humidity (RH) is high and wind speed is low, and conidia release coincides with decreasing humidity and increasing temperature, conditions that generally occur in the morning (28). Viable conidia that are deposited on the leaf of a susceptible lettuce plant germinate and colonize the leaf, resulting in symptoms that are visible 8 to 14 days after the initiation of infection, depending on host susceptibility and environmental conditions (17,19).

Environmental factors such as temperature, RH, wind speed, solar radiation, and leaf wetness duration have been identified as factors that influence sporulation, dispersal, survival, and infection processes (6,20,24,28,29). Hence, conidia survival is greater at 23°C than at 31°C (2 to 5 h) at a RH between 33 and 76%, whereas at a RH ≥ 90%, survival of conidia increases substantially (24,27). Wind speed plays a major role in the conidia dispersal process, and the amount of solar radiation affects the survival of the airborne conidia (4,27). However, the most important factor for successful infection seems to be a duration of morning leaf wetness of at least 3 or 4 h (8,10,16,23). Therefore, many forecasting systems for lettuce downy mildew use morning leaf wetness duration as an indicator for occurrence of an infection period (10,16,18). In fact, according to the forecasting system developed by Scherm et al. (16) (and a modified version developed subsequently), infection by *B. lactucae* occurs on days when leaf wetness ends late in the morning (10:00 hours) (16). The BREMCAST system developed in Quebec, Canada, by Kushalappa (10) considers a leaf wetness duration of 3 to 5 h after dawn (continuing until 10:00 hours) to initiate fungicide application. These systems assume that sporulation is nocturnal, that conidia are released at dawn, and that infections occur in the morning (10,16). However, in a study conducted in Quebec, Carisse and Philion (4) observed conidia release patterns during the afternoon on 21 and 17% of the days when spores were trapped. Moreover, Bhaskara Reddy et al. (2) observed that > 50% of conidia survived a solar radiation dose of 10 MJ/m^2 over a period of 3 h and that on overcast

days, up to 80% of those conidia were viable after 9 h. Wu et al. (30) noted that a forecasting system using a threshold of morning leaf wetness lasting from 06:00 to 10:00 hours missed some infection days, and suggested that a leaf wetness duration of 3 h may be long enough for conidia to germinate. Scherm and van Bruggen (17) also observed some infection under short wetness durations, such as 2 h. Therefore, some major epidemiological questions have arisen from these observations, including the following: What proportion of daily conidia is released in the morning (until 10:00 hours)? Can a large amount of conidia released after 10:00 hours infect lettuce later in the day in the region of Quebec? What is the minimum leaf wetness duration required for infection under field conditions in this region? What are the environmental factors that modulate infection events?

One way of improving lettuce downy mildew forecasting systems is to consider the amount of inoculum available locally, in addition to leaf wetness duration (16). For example, for the oomycete *Phytophthora infestans*, spore sampling coupled with a disease-forecasting system is useful for targeting the optimal time to apply a disease-control products (5,14). For Botrytis leaf blight of onion, which is caused by *Botrytis squamosa*, the use of a fungicide spray program based on a threshold of 10 to 15 conidia/m^3 made it possible to reduce fungicide application by 75 and 56% depending on years and trials (3). Carisse and Philion (4) also observed that fluctuations in the numbers of airborne conidia of *B. lactucae* followed fluctuations in disease severity.

The life cycle of *B. lactucae* can be divided into four distinct stages: sporulation, conidia release, conidia survival, and infection. Each of these processes has been studied independently (10,16,25). However, to study the combined effects of these processes, infection efficiency (IE), defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (ACC), should be considered. Indeed, IE

combines components derived from the pathogen (sporulation and conidia release) with components derived from the host/pathogen interaction (infection of leaves). Therefore, IE is probably influenced by environmental factors such as leaf wetness, solar radiation, and temperature. Despite the large amount of scientific information available on lettuce downy mildew, no field studies have focused on *B. lactucae* IE and the ways in which environmental factors influence IE.

The aim of this research was, therefore, to study the influence of environmental factors on the IE of *B. lactucae* under Quebec weather conditions. Specifically, the study objectives were: (1) to assess the daily percentage of conidia that is released outside the morning period (06:00 to 10:00 hours) and the reliability of using morning leaf wetness duration as a risk indicator under Quebec weather conditions, (2) to examine the relationship between the ACC of *B. lactucae* and the number of lesions/leaf (IE) under growth chamber and field conditions, and (3) to assess the influence of selected weather parameters on IE.

3.3. Materials and Methods

3.3.1. Data collected

3.3.1.1. Pattern of daily airborne conidia concentration (ACC), and relationship between leaf wetness duration and pattern of ACC.

The experiment was conducted between 1 July and 20 September in each of 1997, 1998, 2003, and 2004. Each year, a plot of the lettuce cv. Ithaca was established in

an organic soil at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC (latitude 45°10'N, longitude 73°40'W). Lettuce plants produced in a greenhouse by Les Serres Lefort (Ste-Clothilde, QC, Canada), were transplanted 0.3 m apart in the rows, with 0.35 m between rows. The plots measured 30 m × 30 m, for a total of 33 rows and 5,676 plants. A 7-day volumetric spore sampler (Burkard Manufacturing Co, Rickmansworth, Hertfordshire, UK) placed in the center of the plot was used to monitor ACC (4). The sampler was adjusted to sample air at 10 liters/min. Impaction tapes were coated with a thin layer of silicone grease before they were placed in the sampler. Conidia counts were performed with a microscope at 250× magnification and converted to conidia/ m^3 of air as follows: (number of conidia/rod ×1,000 liters/m^3/h)/(10 liters/min× 60 min/h × 1 h) (4).

3.3.1.2. Relationship between ACC and number of lesions/leaf (IE), and the influence of selected weather parameters on IE.

Growth chamber trial. In a growth chamber (PGC20 growth chamber; Conviron, Winnipeg, MB, Canada), 20 lettuce plants (cv. Ithaca) that had been produced in a greenhouse and had reached the 6- to 8-leaf stage were placed 0.15 m apart in the chamber. Ten lettuce leaves with sporulating lesions were placed 1.25 m above the lettuce plants on a perforated shelf so that the plants were infected by falling airborne conidia in a manner similar to infection under field conditions (Fig.3.1). The amount of inoculum was not controlled over the course of this experiment, but for each trial, an effort was made to provide the same number and size of sporulating lesions. To promote infection, the plants were held for 15 h in the dark, then for 9 h under light (growth chamber fluorescent lamps, 150 umol/m^2/s) at 18°C, and 100% RH, with a leaf wetness period of 6 h. After 24 h, the sporulating leaves were removed, and the growth chamber was maintained at 18°C and 90% RH for 6 days with a daily cycle of 9 h of darkness and 15 h of light. A rotating-arm spore sampler (Compagnie de

Recherche Phytodata, Inc., Sherrington, QC, Canada) placed 0.5 m above the lettuce plants, was used to monitor ACC in the growth chamber. The sampler was operated for 17.6 h/24-h sampling period (alternating between 11 min on and 4 min off), and the effective air-sampling rate was 21 liters/min. Conidia caught on the sampling surfaces (rods) were counted within 24 h after sampling with a microscope at 250× magnification. The number of conidia/rod was converted to conidia/m^3 as follows: (number of conidia/rod ×1,000 liters/m^3/h)/(21 liters/min/rod × 60 min/h × 17.6 h). This experiment was repeated 30 times. The number of lesions/leaf was assessed on all plants in the growth chamber by randomly selecting five leaves on each plant.

Field trials. In each of 2003, 2004, 2012, and 2013, a plot of the lettuce cv. Ithaca was established in an organic soil at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC. Lettuce plants produced in a commercial greenhouse by Les Serres Lefort, were transplanted 0.3 m apart in the rows, with 0.35 m between rows. The plots measured 30 m × 30 m, for a total of 33 rows and 5,676 plants. In the lettuce plots each year 72 lettuce trap plants (cv. Ithaca) were placed randomly in the field each Tuesday, Wednesday, and Thursday for 24 h (total of 216 trap plants/week). The trap plants were divided into four trays (0.135 m x 0.07 m and 0.04 m depth) containing 18 plants each.

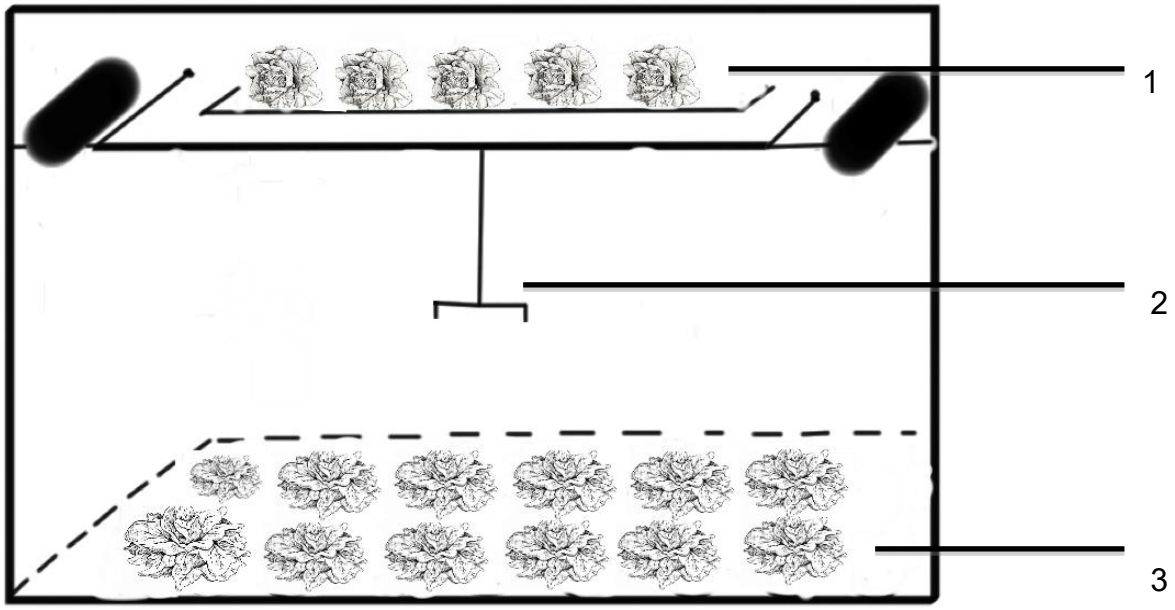

Figure 3. 1. Diagram illustrating the growth chamber set-up for evaluating infection of lettuce plants by the downy mildew pathogen, *Bremia lactucae*: 1, sporulating lettuce leaves, 2, *Bremia lactucae* airborne inoculum sampler (Compagnie de Recherche Phytodata Inc., Sherrington, QC, Canada), and 3, healthy lettuce plants of the cv. Ithaca.

The trap plants placed in the field over the course of the study were always at the same growth stage (5- to 7-leaf stage) and were produced in a greenhouse by Les Serres Lefort. After 24 h in the field of potential exposure to airborne *B. lactucae* inoculum, the trap plants were incubated in a greenhouse for 7 days under 90 % RH and a temperature between 18 and 24°C. After 7 days of incubation, disease severity was assessed as the number of lesions on five randomly selected leaves/plant, excluding leaves that had emerged after exposure in the field. Trap plants were exposed to the potential inoculum release 47 times in 2003, 60 times in 2004, 24 times in 2012, and 23 times in 2013 (for a total of 154 times). While the trap plants were exposed to naturally occurring airborne *B. lactucae* inoculum, ACC was monitored with three rotating-arm spore samplers with retracting sampling heads

(Compagnie de Recherche Phytodata Inc.). Each sampler was supported 1.5 m off the ground by a pole, and the three poles were positioned in the center of the plot in a triangular pattern. Conidia counts were performed with a microscope at 250× magnification and converted to conidia/m^3 of air as follows: (number of conidia/rod × 1,000 liters/m^3/h)/(21 liters/min/rod × 60 min/h × 4.5 h).

3.3.2. Measurement of environmental variables

Leaf wetness duration was assessed every minute by means of an electrical-impedance leaf-wetness sensor (Model 237, Campbell Scientific, Edmonton, AB, Canada) placed at the height of the lettuce leaves in the field trials at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC. Air temperature (°C) and RH (%) were monitored using WatchDog data loggers (Spectrum Technologies, Aurora, IL) located near the spore samplers. Weather variables were monitored every 30 min, and hourly averages were used in the analyses. Temperature and RH probes were placed in a white shelter 1.5 m above the ground. Solar radiation data were obtained from an Environment Canada weather station located approximately 200 m from the plots.

3.3.3. Data analysis

First, normality of the data was tested using the Shapiro–Wilk test, and then, if necessary, logarithmic transformation was done to improve normality. All statistical tests were performed in R version 3.0.0 as described below.

3.3.3.1. Pattern of daily airborne conidia concentration (ACC), and relationship between leaf wetness duration and pattern of ACC

Airborne conidia pattern. Graphical representations of daily ACC released between 06:00 and 10:00 hours as a function of day of the year in 1997, 1998, 2003, and 2004 were prepared. Scherm and van Bruggen (16) observed that *B. lactucae* conidia release in coastal California was initiated at sunrise (06:00 hours) and that infection occurred within a 3- to 4-h leaf wetness duration after sunrise. Each graphical representation for this study shows the daily ACC released during the period of 06:00 to 10:00 hours in comparison with the total daily ACC for a 24-h sampling period, as well as the proportion of daily ACC that was released between 06:00 and 10:00 hours. For each year, the percentage of daily conidia released during and outside the period of 06:00 to 10:00 hours were calculated. The percentages of conidia released between 06:00 and 14:00 hours were also calculated for each year.

Relationship between leaf wetness duration and airborne conidia pattern. A graphical representation of ACC and number of leaf wetness events as a function of time (4-h intervals) was prepared for the data from all 4 years (1997, 1998, 2003, and 2004). A period of at least three consecutive hours of leaf wetness was considered a leaf wetness event (10,16,23). According to the hypothesis of Scherm and van Bruggen (17), regardless of the amount of inoculum, infection occurs on days when leaf wetness ends late in the morning (between 06:00 and 10:00 hours). Thus, time was expressed in 4-h intervals. Fig. 3 shows the number of leaf wetness events in comparison with the ACC during the same period. The percentage of leaf wetness events that occurred between each of 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 hours were also calculated.

To assess the reliability of using a leaf wetness duration of 3 to 4 h as an indicator of infection by *B. lactucae* under Quebec weather conditions, a contingency table was calculated based on a 3-h threshold for leaf wetness duration. Each sampling date (24 h) in 2003 and 2004 was divided into six 4-h periods (02:00 to 06:00, 06:00 to 10:00, 10:00 to 14:00, 14:00 to 18:00, 18:00 to 22:00, and 22:00 to 02:00 hours). For the contingency table, three periods of the day were considered. The period between 06:00 and 10:00 hours was considered the standard in accordance with the Scherm and van Bruggen hypothesis (17). The periods between 10:00 and 14:00 hours and 18:00 and 22:00 hours were also taken into account because the ACC and number of leaf wetness events were considerable during those periods (Fig. 3.3.). For each of these three periods, a leaf wetness duration ≥ 3 h was considered to be a prediction of the presence of lettuce downy mildew (P+) on trap plants, and a leaf wetness duration < 3 h was considered to be a prediction of the absence of this disease (P−). For each sampling day, the presence of downy mildew on trap plants was indicated by O+ (observed), and the absence of disease was indicated by O− (not observed). Therefore, there were four possible outcomes. First, disease was predicted (P+) and observed (O+). This situation was termed a “true positive” (TP). Second, disease was not predicted (P−) and was not observed (O−), termed a “true negative” (TN). Third, disease was predicted (P+) but was not observed (O−), termed a “false positive” (FP). Fourth, disease was not predicted (P−) but was observed (O+), termed a “false negative” (FN). The proportion of true positives was considered the indicator sensitivity [TP/(TP + FN)]. The proportion of true negatives was considered the indicator specificity [TN/(TN + FP)]. Overall accuracy was calculated as the number of correct assessments (TN + TP) divided by the number of all assessments (TP + TN + FP + FN). The likelihood ratio of positive predictions (LR+) was estimated by LR+ = sensitivity/(1-specificity), and the likelihood ratio of negative predictions (LR-) was estimated by LR- = (1-sensitivity)/specificity (9,12).

3.3.3.2. Relationship between ACC and number of lesions/leaf

Growth chamber trial. A scatter plot was prepared to represent the number of lesions/leaf as a function of ACC. A linear function was fitted to the data, as follows:

$$y = \beta_0 + \beta_1 x \quad (1)$$

where y is the number of lesions/leaf, χ is the ACC, β_0 is the intercept parameter, and β_1 is the slope.

Field trial. Scatter plots representing the number of lesions/leaf as a function of ACC were prepared for the data from each of 2003, 2004, 2012, and 2013, and for the data from all four of these years. A three-parameter sigmoid model was fitted to these data as follows:

$$y = \frac{a}{1 + e^{-(\frac{x - x_0}{b})}} \quad (2)$$

where y is the number of lesions/leaf, a is the asymptote, x_0 is the ACC at the inflection point where the number of lesions/leaf is 50% of the asymptote, and b is the slope of the linear portion of the curve. The model appropriateness and goodness of fit to the data were assessed using an adjusted R^2 and a concordance correlation coefficient (CCC) (12).

3.3.3.3. Relationship between IE and weather parameters under field conditions

Correlations among IE and selected weather variables were analyzed using Spearman's rank correlation matrix in R (Hmisc package 3.4.0). For each sampling date, the average temperature (TPa), maximum solar radiation (SR), and leaf wetness duration (LWD) were considered.

Binary recursive partitioning in the R tree package was used to build a classification tree for IE (15). This process successively split the data along coordinate axes of explanatory variables so that, at any node, the split which maximally distinguished the response variable in the left and right branches, was selected. Each explanatory variable (TPa, SR, and LWD) was assessed in turn, and the variable explaining the greatest amount of deviance in IE was selected. The smallest permitted node was 10, and the node was not partitioned if the within deviance was ≤ 0.01 of the root node (15). The two explanatory variables explaining the greatest amount of deviance in IE were used to plot the classification tree using the *partition.tree* function. To construct the partition plot, IE was transformed into a binary variable where IE values of ≥ 0.1 (1 lesion/leaf/10 conidia/m^3) were coded as "ls" (lesion) or "nls" (no lesion). Infection efficiency values between 0.056 and 0.099 were rounded to 0.1.

3.4. Results

3.4.1 Pattern of daily airborne conidia concentration (ACC), and relationship between leaf wetness duration and pattern of ACC

Most conidia were released outside the period between 06:00 and 10:00 hours (Fig. 3.2. and 3.3.). On 91, 95, 90, and 92% of the sampling days, conidia released between 06:00 and 10:00 hours accounted for < 50% of the conidia released daily in 1997, 1998, 2003, and 2004, respectively (Fig. 3.2.). The maximum ACC was 12,236, 2,136, 2,885, and 3,707 in 1997, 1998, 2003, and 2004, respectively (Fig. 3.2.).

The percentage of conidia released between 06:00 and 14:00 hours was 70, 76, 73, and 71% of the total conidia released daily in 1997, 1998, 2003, and 2004, respectively (Fig. 3.3.). In 1997, 76.6, 6.4, and 17.0 % of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 hours, respectively (Fig. 3.3.). In 1998, 90, 3.3, and 6.7% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 hours, respectively. In 2003, 79, 14, and 7% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 hours, respectively. In 2004, 79, 6.5, and 14.5% of all leaf wetness events occurred between 22:00 and 06:00, 06:00 and 10:00, and 10:00 and 22:00 hours, respectively.

According to the data from all four years together, 80% of all leaf wetness events occurred between 22:00 and 06:00 hours, 7% between 06:00 and 10:00 hours, 13% between 10:00 and 22:00 hours, and 8.5% between 18:00 and 22:00 hours. In addition, the greatest daily ACCs were detected between 06:00 and 14:00 hours

(Fig. 3.3.). The overall accuracy of using a leaf wetness duration ≥3 h as a lettuce downy mildew risk indicator was greater when morning (06:00 to 10:00 hours) leaf wetness was used than when evening (18:00 to 22:00 hours) leaf wetness was used (accuracy of 0.545 vs. 0.382). Using only morning leaf wetness led to 39.5% of downy mildew infections being predicted (sensitivity = 0.395).

In contrast, using both morning and evening leaf wetness as a downy mildew risk indicator led to 68.4% of downy mildew infections being predicted. Also, using both morning and evening leaf wetness did not increase the likelihood ratio of positive prediction of downy mildew but decreased the likelihood ratio of negative prediction of downy mildew (Table 3.1.). However, using leaf wetness events between 10:00 and 14:00 hours (T2 in Table 3.1.), morning leaf wetness events (between 06:00 and 10:00 hours, T1, Table 1), and evening leaf wetness events (between 18:00 and 22:00 hours, T3 in Table 3.1.) did not improve the accuracy and sensitivity of the downy mildew risk indicator compared to using the combination of morning leaf wetness and evening leaf wetness (T1 + T3 in Table 3.1.).

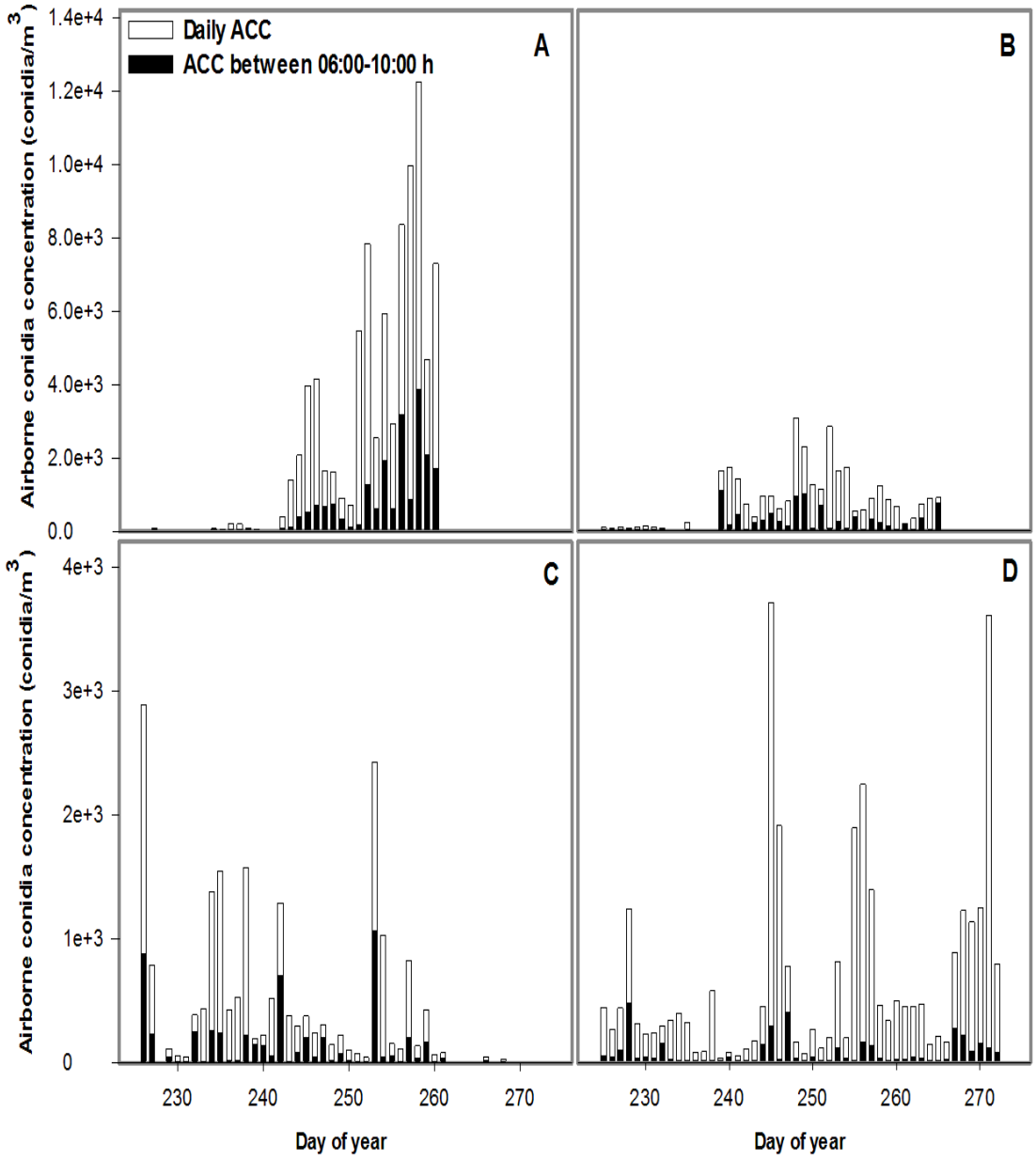

Figure 3.2. Daily airborne conidia concentration (ACC) of *Bremia lactucae* as a function of day of the year (where day 1 is 1 January) in A, 1997, B, 1998, C, 2003, and D, 2004. The term "Total daily ACC" represents the ACC for a 24-h sampling period. This study was done in lettuce crops at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, Quebec, Canada.

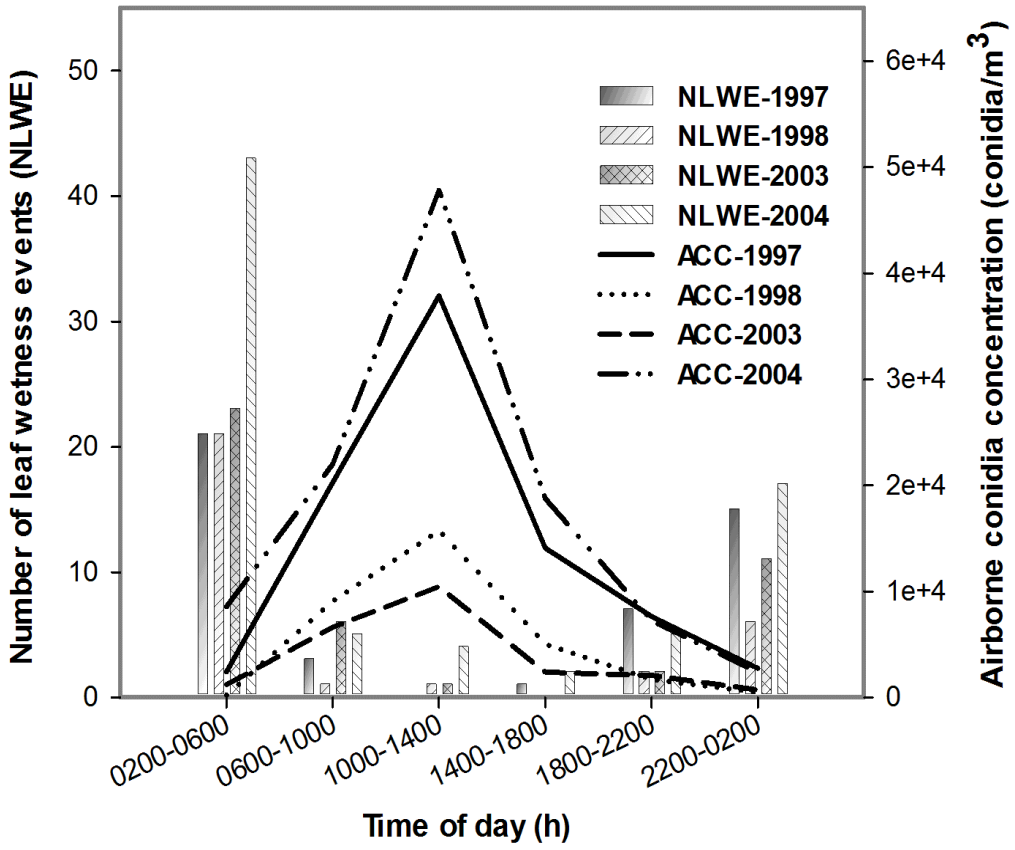

Figure 3.3. Number of lettuce leaf wetness events (NLWE) and *Bremia lactucae* airborne conidia concentration (ACC) as a function of 4-h intervals. A period of at least three consecutive hours of leaf wetness was considered a leaf wetness event (10, 16, 24). This study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, in Quebec, Canada in 1997, 1998, 2003, and 2004.

Table 3.1. Contingency table for assessment of the reliability of using a leaf wetness duration of 3 to 4 h as an indicator of infection of lettuce plants by *Bremia lactucae* under field conditions in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada

Infection period[a]	Observed	Predicted		Total	Overall accuracy[b]	Sensitivity[b]	Specificity[b]	LR+ [c]	LR- [c]
		P+	P−						
06:00–10:00 hours (T1)	O+	15	23	38	0.545	0.395	0.882	3.00	0.69
	O−	2	15	17					
	Total	17	38	55					
10:00–14:00 hours (T2)	O+	2	36	38	0.317	0.053	0.941	0.89	1.01
	O−	1	16	17					
	Total	3	52	55					
18:00–22:00 hours (T3)	O+	5	33	38	0.382	0.132	0.941	2.24	0.92
	O−	1	16	17					
	Total	6	49	55					
T1 + T3	O+	26	12	38	0.709	0.684	0.765	2.91	0.41
	O−	4	13	17					
	Total	30	25	55					
	O+	26	12	38					
	O−	4	13	17					
	Total	30	25	55					

[a] For each of the infection periods T1, T2, T3, and T1 + T3, a leaf wetness duration of ≥ 3 h was considered to be a prediction of the presence of downy mildew (P+) on lettuce trap plants, and a leaf wetness duration < 3 h was considered a prediction of the absence of downy mildew (P−). For each sampling day, the presence of lesions on trap plants was indicated by O+ (observed), and the absence of lesions was indicated by O− (not observed).

[b] The proportion of true positives (O+ and P+) was regarded as a measure of sensitivity, the proportion of true negatives (O- and P−) as specificity, and the overall accuracy was calculated as the number of correct assessments (O+ and P+, and O- and P−) divided by the number of all assessments (9).

[c] LR+ and LR- = positive likelihood ratio and negative likelihood ratio, respectively, LR+ = sensitivity/(1-specificity) and, LR- = (1-sensitivity)/specificity (12).

3.4.2. Relationship between ACC and number of lesions/leaf

Under growth chamber conditions, the number of lesions/leaf was directly proportional to ACC (Fig. 3.4.). An ACC of 1 conidium/m^3 was sufficient to cause 1 lesion/leaf based on the regression equation shown on Fig. 3.4.

Under field conditions, x_0, which is an estimate of the ACC at the inflection point that corresponds to the number of lesions/leaf at 50% of the maximum (asymptote), was 122.80 ± 3.85 (standard error), 117.46 ±3.35, 118.33 ± 6.97, and 121.62 ± 6.80 conidia/m^3 in 2003, 2004, 2012, and 2013, respectively (Table 3.2., Fig. 3.5.). When the data from all four years were pooled, 116.75 ± 2.43 conidia/m^3 were required to achieve 50% of the maximum number of lesions/leaf (20 lesions) (Table 3.2., Fig. 3.6.). According to a sigmoidal regression model (Fig. 3.6.), an ACC of 14 conidia/m^3 was sufficient to cause 1 lesion/leaf. The sigmoidal model for all four years in Fig. 3.6. consisted of three phases: a quasi-linear phase between 0 and 15 lesions/leaf, in which the number of lesions/leaf was proportional to the ACC; a deceleration phase between 15 and 20 lesions/leaf; and a stationary phase of about 20 lesions/leaf, in which the number of lesions did not change even though the ACC increased (Fig. 3.6.).

3.4.3. Relationship between IE and selected weather parameters

Under controlled conditions in the growth chamber trials, IE ranged from 0.250 to 1.000, whereas under field conditions, IE ranged from 0.034 to 0.100 in 2003, 0.033 to 1.000 in 2004, 0.000 to 0.100 in 2012, and 0.000 to 0.023 in 2013 (Fig. 3.7.). Some weather parameters had significant associations with *B. lactucae* IE under field

conditions. For all four years, IE increased as LWD increased (Table 3.3.), whereas for all years except 2003, IE decreased as daily Tpa increased; in 2003, there was no significant association between the TPa and IE (Table 3.3.). Overall, the Tpa association with IE was weak (correlation coefficients of 0.00, -0.06, -0.62, and -0.06 in 2003, 2004, 2012, and 2013, respectively), and SR did not have a significant association with IE except in 2004 (correlation coefficient was -0.60 and P = 0.04) (Table 3.3.). LWD was associated significantly with IE measured in all years, and Tpa had limited association with IE (Table 3.3, Fig. 3.8.).

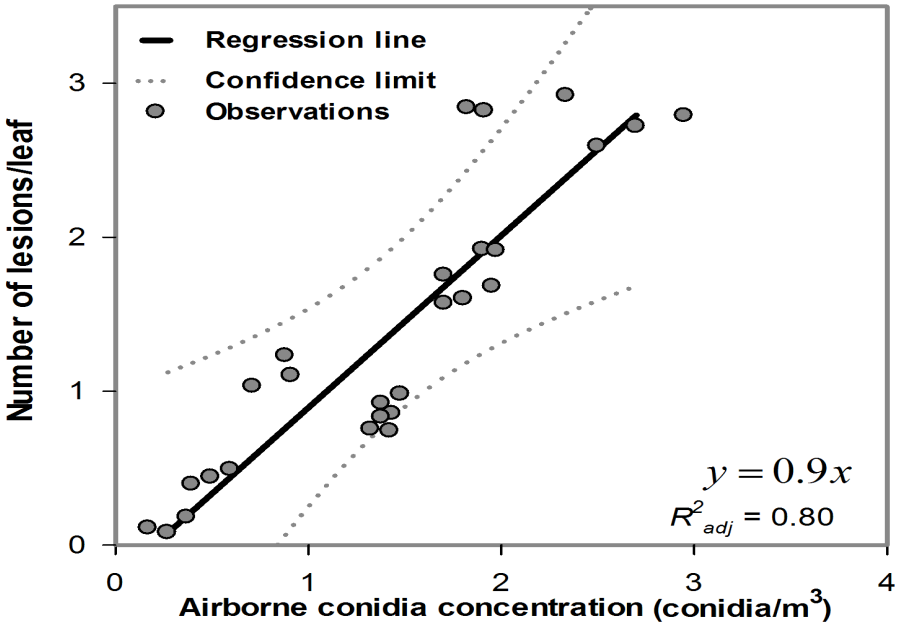

Figure 3.4. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under controlled conditions in a growth chamber (refer to main text for details on growth chamber conditions and lettuce inoculation protocol). This study was done in Quebec, Canada. The solid straight line represents the fitted linear function and dotted lines represent the confidence interval.

Table 3.2. Estimated three-parameter sigmoidal regression models describing the relationship between the number of downy mildew lesions/leaf and *Bremia lactucae* airborne conidia concentration under field conditions in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada

Year	a[a]	P[b]	b[c]	P[d]	x_0[e]	P[f]
2003	22.96 (0.80)	<0.0001	39.76 (2.03)	<0.0001	122.80 (3.85)	<0.0001
2004	20.24 (0.48)	<0.0001	35.46 (2.14)	<0.0001	117.47 (3.35)	<0.0001
2012	12.39 (3.16)	0.0008	18.99 (4.78)	0.0007	118.34 (6.97)	<0.0001
2013	15.73 (0.38)	<0.0001	71.43 (6.45)	<0.0001	121.62 (6.80)	<0.0001
2003 + 2004 + 2012 + 2013	19.29 (0.36)	<0.0001	31.42 (1.60)	<0.0001	116.75 (2.43)	<0.0001

[a] *a* = the asymptote of the three-parameter sigmoid model, and the number in parentheses = standard error.
[b] *P* = the probability that *a* is significantly different from 0 at α = 0.05.
[c] *b* = the slope of the linear portion of the curve.
[d] *P* = the probability that *b* is significantly different from 0 at α = 0.05.
[e] x_0 = airborne conidia concentration at the inflection point where the number of lesions/leaf = 50% of the asymptote.
[f] *P* = probability that x_0 is significantly different from 0 at α = 0.05.

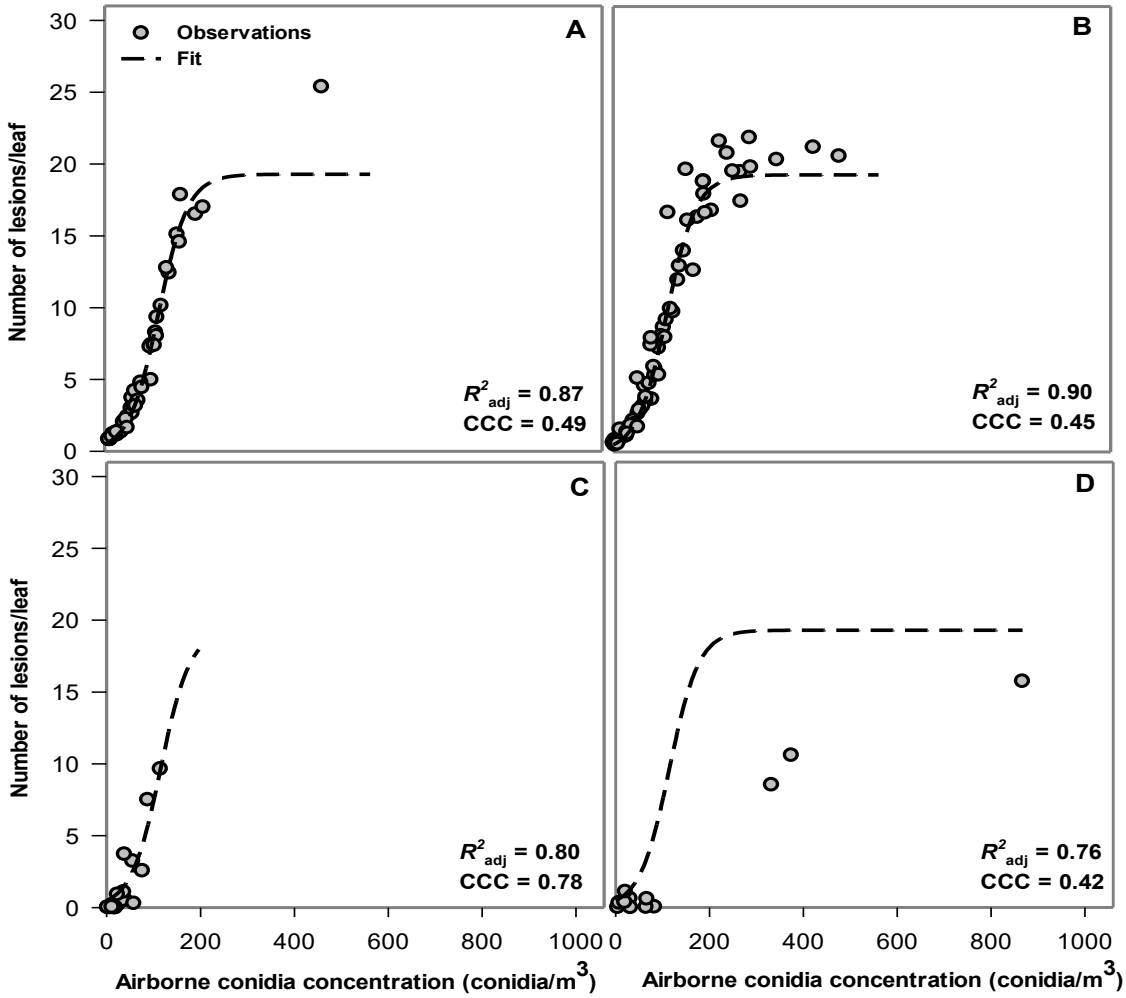

Figure 3.5. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under field conditions in A, 2003, B, 2004, C, 2012, and D, 2013. A three-parameter sigmoidal model was fitted with the same shape parameter for each year (See Equation 2 in the main text). CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12). R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada. Each data point represents the mean number of lesions/leaf and the airborne conidia concentration.

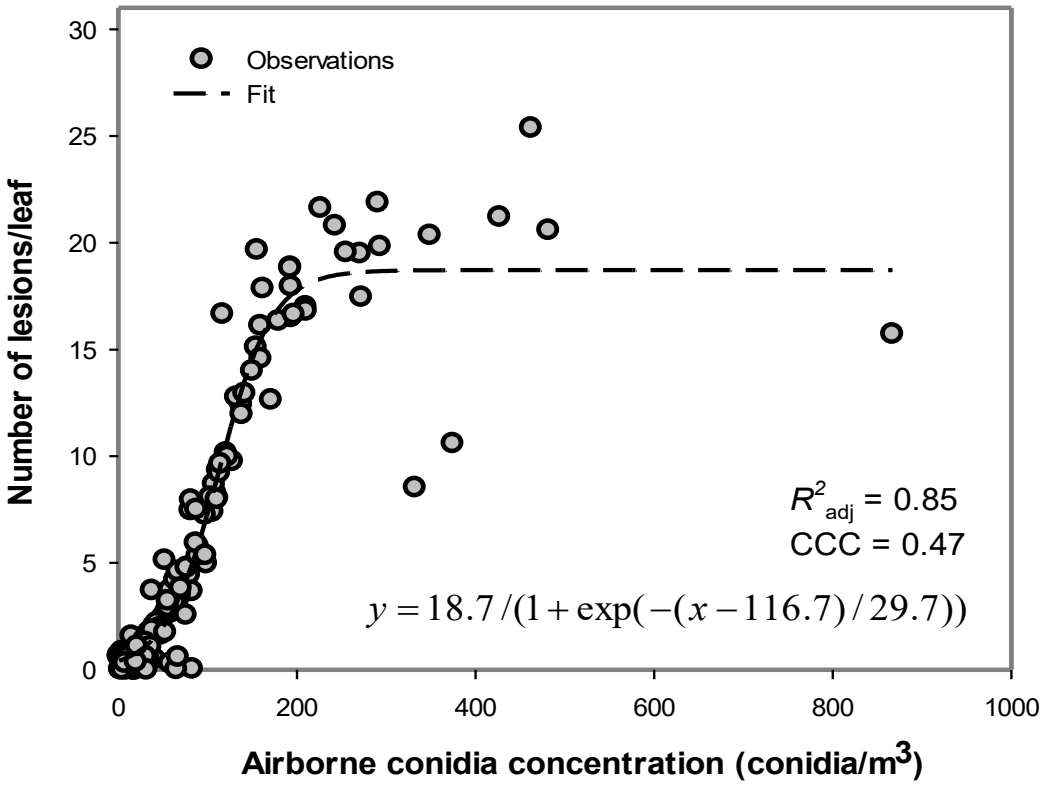

Figure 3.6. Relationship between the number of downy mildew lesions/lettuce leaf and *Bremia lactucae* airborne conidia concentration under field conditions for the data pooled from four years (2003, 2004, 2012, and 2013). A three-parameter sigmoid model was fitted to the data (see Equation 2 in the main text). CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12). R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada. Each data point represents the mean number of lesions/leaf and the airborne conidia concentration.

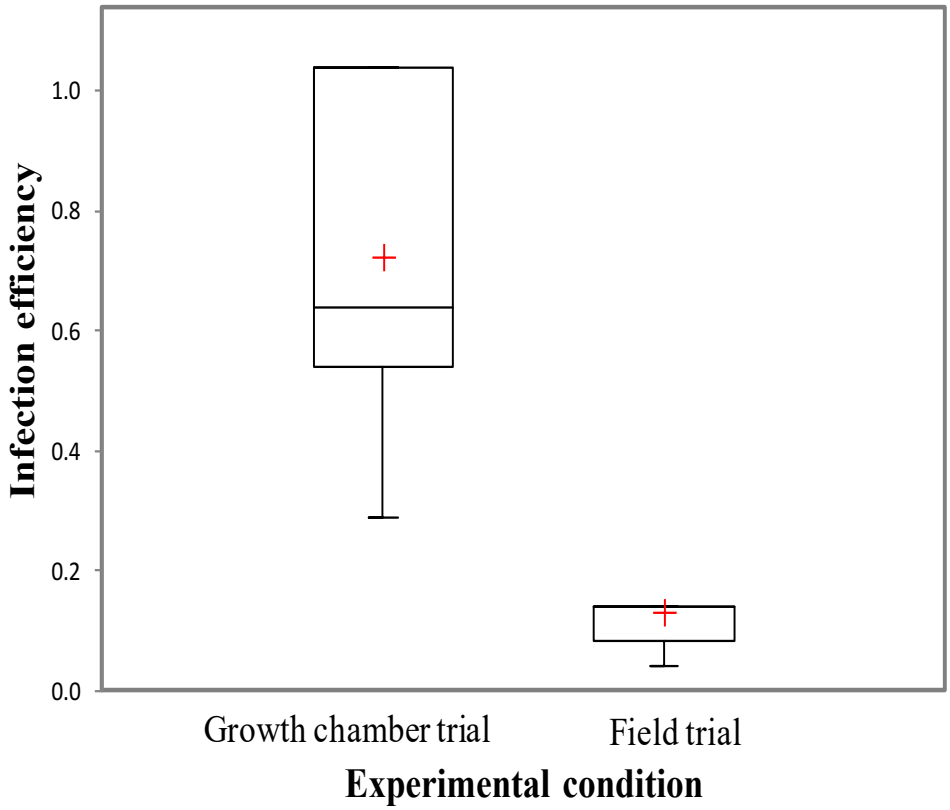

Figure 3.7. *Bremia lactucae* infection efficiency (IE) on plants of the lettuce cv. Ithaca under growth chamber conditions and field conditions at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada, with *n* = 30 plants in the growth chamber trials, and *n* = 154 for trap plants in the field trials. Infection efficiency (IE), was defined as the ratio of the number of lesions/leaf to the airborne conidia concentration (ACC). Refer to the main text for details on the growth chamber conditions and the lettuce inoculation protocol. The "whiskers" of the box plot represent the lower quartile, "+" sign represents the mean value, and horizontal line inside box plot represents the median value.

Table 3.3. Correlation matrix for infection efficiency (IE) of the lettuce downy mildew pathogen, *Bremia lactucae*, average daily air temperature (TPa), solar radiation (SR), and leaf wetness duration (LWD). This study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada

Year	Variable[a]	TPa [c] (°C)	SR[d] (KJ/m^2)	LWD[e] (min)
	IE[b]	0.00	−0.03	0.76***[f]
2003	LWD	0.00	−0.17	
	SR	0.26		
	IE	−0.06*[f]	0.25	0.66***
2004	LWD	−0.05*	0.25	
	SR	0.04		
	IE	−0.62*	−0.60*	0.72***
2012	LWD	−0.28**[f]	−0.49	
	SR	0.36		
	IE	−0.06*	−0.21	0.68***
2013	LWD	−0.39	−0.35	
	SR	−0.04*		
	IE	−0.48*	0.19	0.84***
2003 + 2004 + 2012 + 2013	LWD	−0.33	0.06	
	SR	−0.16*		

[a] Refer to main text for details on measurement of the variables TPa, SR, and LWD.
[b], IE = infection efficiency, defined as the ratio of the number of lesions/leaf to the airborne conidia
concentration (ACC)
[c], Tpa = average daily air temperature.
[d], SR = solar radiation .
[e], LWD = lettuce leaf wetness duration .
[f], *** = $P < 0.001$; ** = $P < 0.01$; * = $P < 0.05$.

There was an exponential relationship between IE and LWD, with 120 min (2 h) of leaf wetness being sufficient to cause an IE of 0.1 (Fig. 3.8.). Under field conditions, 2 h of LWD and an ACC of 10 conidia/m^3 were needed to cause 1 lesion/leaf as long as the Tpa was < 20°C (Fig. 3.9.). A leaf wetness duration of ≥ 180 min (3 h) caused

at least 1 lesion/leaf even at temperatures between 20 and 25°C. Temperatures ≥ 26°C were not tested in this study (Fig. 3.9).

3.5. Discussion

The majority of Canadian lettuce production is located in the province of Quebec (1), and each year, downy mildew caused by *B. lactucae* occurs in this area (4). Yet information on the epidemiology of *B. lactucae* is derived primarily from controlled-condition experiments or field experiments conducted in subtropical climates and, therefore, cannot readily be applied to Quebec lettuce production (2,13,17,18,19,20,23,24,27). Even though considerable efforts have been made to develop downy mildew resistant lettuce cultivars, no cultivar is resistant at all growth stages (seeding, young plant, and mature plant) and to all races of *B. lactucae* to avoid losses for growers (22). Therefore, efficient control of lettuce downy mildew currently depends on a better understanding of the epidemiology of *B. lactucae* and on implementing appropriate control measures. Moreover, lettuce is a short-season crop (45 to 55 days in Quebec), and on farms with successive plantings of lettuce each season, as in Quebec, the potential risk of downy mildew and consequent yield losses is affected by inoculum build-up over the season. The main purpose of this study was, therefore, to investigate the relationship between *B. lactucae* airborne inoculum and downy mildew severity under Quebec weather conditions, and the ways in which environmental factors influence this relationship.

In Quebec lettuce production systems, the assumption that infection by *B. lactucae* occurs only between 06:00 and 10:00 hours as a result of studies on the disease in other climates and regions, has meant that > 50% of the conidia released daily have not been considered. These conidia would need only to survive until the next leaf

wetness event to infect lettuce leaves. Thus, under favorable weather conditions, there is a strong probability that infection can occur after 10:00 hours. In this context, favorable conditions refer to leaf wetness, which is the most important variable associated with lettuce downy mildew infection (10,20). According to data analyzed in this study for 1997, 1998, 2003, and 2004, 80% of leaf wetness events occurred between 22:00 and 06:00 hours, 7% between 06:00 and 10:00 hours, and 13% between 10:00 and 22:00 hours. Thus, infection by *B. lactucae* could occur at any time of the day. Also, using only morning leaf wetness as a lettuce downy mildew risk indicator led to 60% of downy mildew infections being missed. To improve the accuracy and sensitivity of leaf wetness events as a risk indicator of lettuce downy mildew, both morning and evening leaf wetness events should be considered. The results also suggest that, under Quebec weather conditions, infection by *B. lactucae* occur mostly during the morning and evening. Similar observations have been made in subtropical climates where conidia were released mostly at dawn, and leaves were infected primarily in the mornings (10,16). However, Hovius et al. (8) observed that under Ontario (Canada) weather conditions, lettuce downy mildew did not always develop despite multiple leaf wetness events. Therefore, the limiting factor might be not only leaf wetness duration but also ACC. Scherm et al. (16) suggested that the availability of inoculum must be considered for predicting lettuce downy mildew. Hence, in addition to LWD, ACC should be considered, provided that the relationship between ACC and the severity of infection is known.

Under controlled growth chamber conditions in this study, the number of lesions/leaf was directly proportional to ACC, and an ACC of 1 conidium/m^3 was sufficient to cause 1 lesion/leaf. Under field conditions, however, the number of lesions/leaf was proportional to ACC up to 15 lesions/leaf, and the maximum number of lesions observed/leaf was 20, probably reflecting the limited amount of healthy leaf area available at such inoculum density. An ACC of 14 conidia/m^3 was required to cause 1 lesion/leaf in the field. For Botrytis leaf blight of onion, the number of

lesions/leaf was directly proportional to *B. squamosa* ACC, and a threshold of 10 to 15 conidia/m^3 was used successfully to rationalize fungicide applications (3,26). Similarly, the results of this study suggest that a *B. lactucae* ACC of 14 conidia/m^3 may be a threshold to time fungicide applications for lettuce downy mildew management in this region. However, to improve the reliability of this threshold, it will be imperative to understand how environmental factors influence infection of lettuce by *B. lactucae*, for which the IE should provide a reliable response.

In this study IE increased with increasing LWD, but decreased with increasing Tpa, although the Tpa association with IE was weak (correlation coefficients of 0.00, -0.06,-0.62, and -0.06 in 2003, 2004, 2012 and 2013, respectively). These results are supported by the findings of Scherm and van Bruggen (17,19) and Wu et al. (27,29), who reported that an increase in infection by *B. lactucae* resulted from increasing LWD. Wu et al. (27) also concluded that the effect of temperature was minor. Moreover, this study found that the amount of SR recorded in the Quebec region did not have a significant association with IE, except in 2004 (P= 0.04). These results differ from results reported by Wu et al. (27), who found that a dose of 0.5 MJ/m^2 was sufficient to kill most of *B. lactucae* conidia, whereas the SR registered over the course of this study ranged from 0.5 and 3.8 MJ/m^2. Similarly, Bhaskara Reddy et al. (2) found that ≥ 50% of *B. lactucae* spores survived a SR dose of 10 MJ/m^2. In summary, LWD seemed to explain most of the variation in IE, and Tpa had a minor effect on IE.

The IE of *B. lactucae* in this study increased exponentially with increasing LWD. To achieve an IE of 0.1 (1 lesion/10 conidia/m^3), 120 min of leaf wetness was sufficient. Thus, at an average temperature of 20°C, 2 h of leaf wetness was sufficient to cause infection by *B. lactucae*. These results diverge from those reported by Scherm and van Bruggen (19), who established a minimum LWD of 4 h at temperatures between

5 and 20°C for successful infection in California. Therefore, in Quebec lettuce production systems, it may not be sufficient to consider a LWD of 3 to 5 h after dawn as a lettuce downy mildew risk indicator to initiate fungicide applications. Moreover, a LWD of > 180 min enabled at least 1 lesion/leaf to develop, even at temperatures between 20 and 26°C.

This study examined the relationship between the ACC of *B. lactucae*, the number of lesions/leaf, and selected weather parameters that may significantly influence IE. The results showed that under controlled conditions, an ACC of 1 conidium/m^3 was sufficient to cause 1 lesion/leaf, whereas under field conditions, an ACC of 10 to 14 conidia/m^3 was required to cause the same disease severity. Consequently, in addition to monitoring leaf wetness periods, real-time monitoring of ACC should help improve lettuce downy mildew control measures for growers. Infection by *B. lactucae* can occur at any time of the day if there is a leaf wetness duration of ≥ 2 h. This study also revealed that, under Quebec weather conditions, both morning and evening leaf wetness events should be used to improve the reliability of LWD as a lettuce downy mildew risk indicator.

To the authors' knowledge, this is the first study to focus on the IE of *B. lactucae* under field conditions, and how environmental factors can influence EI. This study lays the groundwork for future use of an ACC of 10 to 14 conidia/m^3 and a LWD of 2 h as risk indicators for determining the optimal time to apply downy mildew fungicide. In the Quebec lettuce production area, airborne conidia of *B. lactucae* could be monitored seasonally, to assess the usefulness of these risk indicators. A similar risk indicator has been used successfully in onion crops in the same area to manage Botrytis leaf blight (3). Nevertheless, further research should be done to validate these risk indicators and assess how they can be integrated into management strategies for lettuce downy mildew in this region.

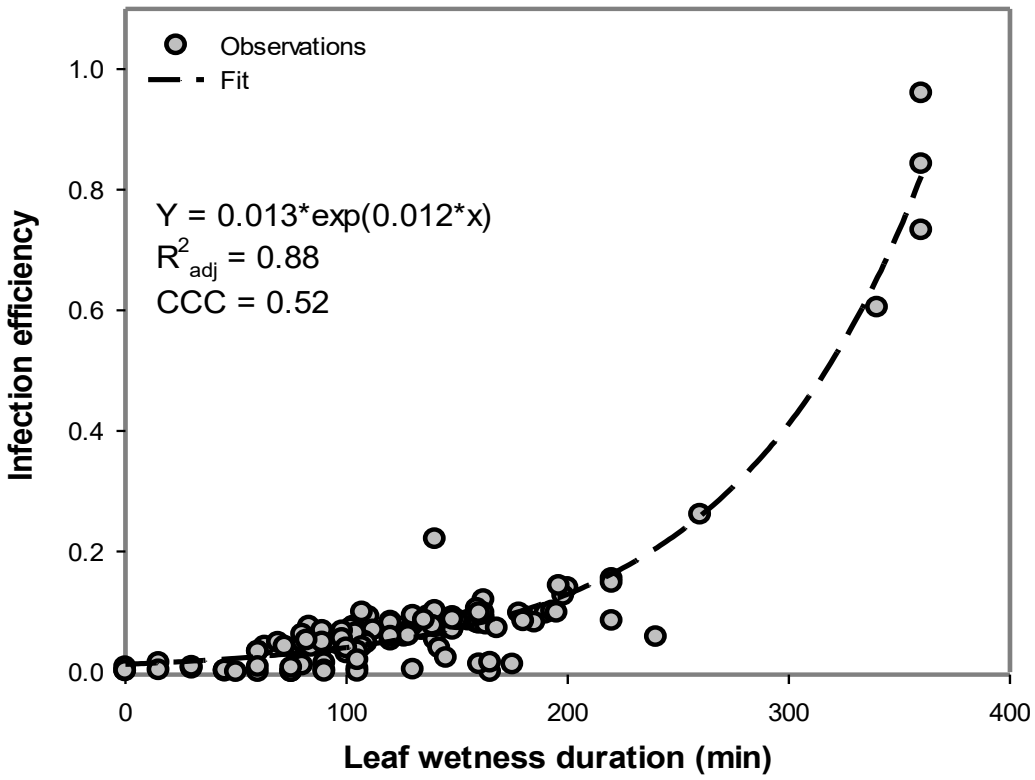

Figure 3.8. Relationship between *Bremia lactucae* infection efficiency (IE) and lettuce leaf wetness duration under field conditions for the data pooled from four years (2003, 2004, 2012, and 2013) together. An exponential growth function was fit to the data. IE was defined as the ratio between the number of downy mildew lesions/leaf and airborne conidia concentration (ACC). CCC = concordance correlation coefficient, a measure of model appropriateness and goodness of fit (12). R^2_{adj} = adjusted coefficient of determination. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada. Each data point represents the mean number of lesions/leaf and the ACC.

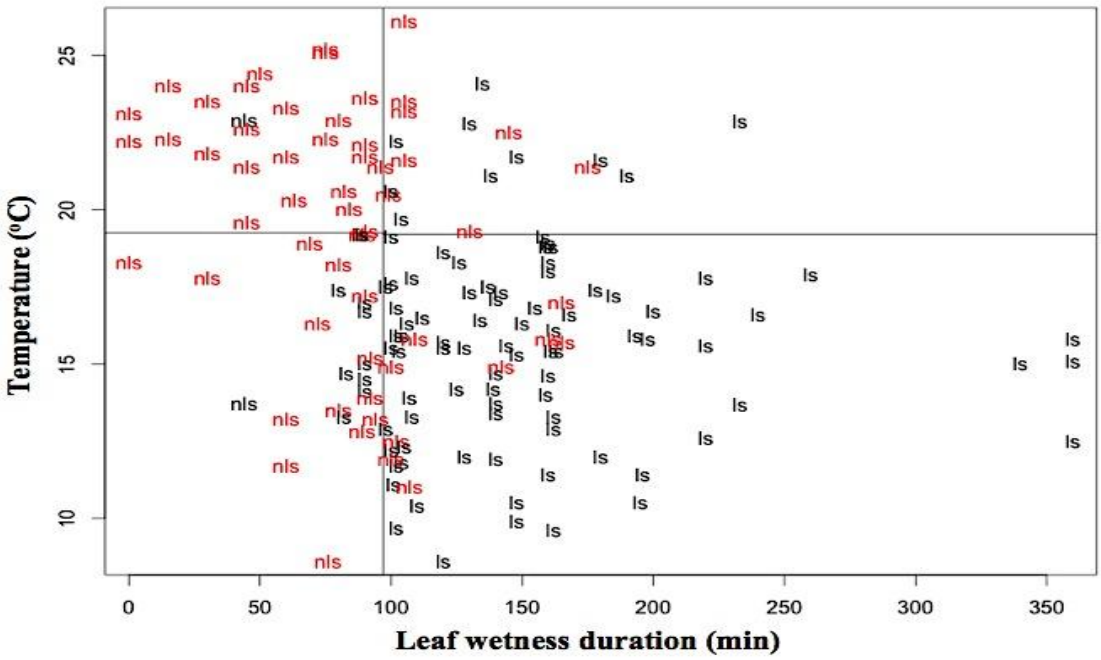

Figure 3.9. The occurrence of lettuce downy mildew (*Bremi lactucae*) lesions (ls) and lack of lesions (nls = no-lesion events) based on leaf wetness duration and average daily air temperature. The data from four years (2003, 2004, 2012, and 2013) were pooled. *B. lactucae* infection efficiency (IE) was defined as the ratio of the number of lesions/leaf to airborne conidia concentration (ACC). IE was transformed to a binary variable where IE values ≥ 0.100 (1 lesion/leaf/10 conidia/m^3) were coded as "ls" (lesion) and IE values ≤ 0.055 coded as "nls" (no lesion). IE values between 0.056 and 0.099 were rounded to 0.1. The study was done in a field at the Agriculture and Agri-Food Canada experimental farm in Ste-Clothilde, Quebec, Canada, with *n* = 154 trap plants in each field trial. The vertical line represents a threshold based on the leaf wetness duration, which distinguished the lesions events (ls) and lack of lesion events (nls). The horizontal line represents a threshold based on the average daily temperature, which distinguished the lesions events (ls) and lack of lesion events (nls).

3.6. Acknowledgments

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3.7. Literature Cited

Anonymous. 2013. Area, production and farm gate value of vegetables, annual. Statistics Canada Table 001-0013. Online publication. Retrieved 20 February 2014 from http://www5.statcan.gc.ca/cansim/a05?lang=eng&id=0010013&pattern=0010013&searchTypeByValue=1&p2=35.

Bhaskara Reddy, M. V., Kushalappa, A. C., and Stephenson, M. M. P. 1996. Effect of solar radiation on the survival of Bremia lactucae spores on lettuce. Phytoprotection 77, 137 (Abst.).

Carisse, O., McCartney, H. A., Gagnon, J. A., and Brodeur, L. 2005. Quantification of airborne inoculum as an aid in the management of leaf blight of onion caused by Botrytis squamosa. Plant Dis. 89:726-733.

Carisse, O., and Philion, V. 2002. Meteorological factors affecting periodicity and concentration of airborne spores of Bremia lactucae. Can. J. Plant Pathol. 24:184-193.

Fall, M. L., Van der Heyden, H., Brodeur, L., Leclerc, Y., Moreau, G., and Carisse, O. 2014. Spatiotemporal variation in airborne sporangia of Phytophthora infestans: Characterization and initiative toward improving potato late blight risk estimation. Plant Pathol. In press. doi:10.1111/ppa.12235.

Fletcher, J. T. 1976. Bremia lactucae, oospores, sporangial dissemination, and control. Ann. Appl. Biol. 84:294-298.

Heffer Link, V., Powelson, M. L., and Johnson, K. B. 2002. Oomycetes. In: The Plant Health Instructor. American Phytopathological Society, Education. Online publication. doi:10.1094/PHI-I-2002-0225-01.

Hovius, M. H. Y., McDonald, M. R., Carisse, O., and Philion, V. 2007. Field evaluation of weather-based spray programs for the control of downy mildew of lettuce (Lactuca sativa), caused by Bremia lactucae, in Quebec and Ontario. Can. J. Plant Pathol. 29:9-17.

Hughes, G. 2011. Applications of Information Theory to Epidemiology. American Phytopathological Society, St. Paul, MN. 221 Pp.

Kushalappa, A. C. 2001. BREMCAST: Development of a system to forecast risk levels of downy mildew on lettuce (Bremia lactucae). Int. J. Pest Manage. 47:1-5.

Lamour, K., and Kamoun, S. 2009. Oomycete Genetics and Genomics: Diversity, Interactions and Research Tools. Wiley-Blackwell, John Wiley & Sons, Hoboken, NJ. 582 Pp.

Madden, L. V., Hughes, G., and van den Bosch, F. 2007. The Study of Plant Disease Epidemics. American Phytopathological Society, St. Paul, MN. 432 Pp.

Nordskog, B., Gadoury, D. M., Seem, R. C., and Hermansen, A. 2007. Impact of diurnal periodicity, temperature, and light on sporulation of Bremia lactucae. Phytopathology 97:979-986.

Olanya, O. M., Honeycutt, C. W., Tschöepe, B., Kleinhenz, B., Lambert, D. H., and Johnson, S. B. 2012. Effectiveness of SIMBLIGHT1 and SIMPHYT1 models for predicting Phytophthora infestans in the north-eastern United States. Arch. Phytopathol. Plant Protect. 45:1558-1569.

Ripley, B. 2014. Classification and regression trees version 1.0-35. R packages. The Comprehensive R Archive Network (cran). Online publication. Retrieved 10 February 2014.

Scherm, H., Koike, S. T., Laemmlen, F. F., and van Bruggen, A. H. C. 1995. Field evaluation of fungicide spray advisories against lettuce downy mildew (Bremia lactucae) based on measured or forecast morning leaf wetness. Plant Dis. 79:511-516.

Scherm, H., and van Bruggen, A. H. C. 1993. Response surface models for germination and infection of Bremia lactucae, the fungus causing downy mildew of lettuce. Ecol. Modell. 65:281-296.

Scherm, H., and van Bruggen, A. H. C. 1993. Sensitivity of simulated dew duration to meteorological variations in different climatic regions of California. Agric. For. Meteorol. 66:229-245.

Scherm, H., and van Bruggen, A. H. C. 1994. Effects of fluctuating temperatures on the latent period of lettuce downy mildew (Bremia lactucae). Phytopathology 84:853-859.

Scherm, H., and van Bruggen, A. H. C. 1994. Weather variables associated with infection of lettuce by downy mildew (Bremia lactucae) in coastal California. Phytopathology 84:860-865.

Shurtleff, M. C., and Averre III, C. W. 1997. Glossary of Plant-Pathological Terms. American Phytopathological Society, St. Paul, MN. 361 Pp.

Simko, I., Atallah, A. J., Ochoa, O. E., Antonise, R., Galeano, C. H., Truco, M. J., and Michelmore, R. W. 2013. Identification of QTLs conferring resistance to downy mildew in legacy cultivars of lettuce. Sci. Rep. 3: 2875. Online publication. doi:10.1038/srep02875.

Su, H., van Bruggen, A. H. C., and Subbarao, K. V. 2000. Spore release of Bremia lactucae on lettuce is affected by timing of light initiation and decrease in relative humidity. Phytopathology 90:67-71.

Su, H., van Bruggen, A. H. C., Subbarao, K. V., and Scherm, H. 2004. Sporulation of Bremia lactucae affected by temperature, relative humidity, and wind in controlled conditions. Phytopathology 94:396-401.

Tchervenivanova, E. 1995. Development of a model to predict sporulation of Bremia lactucae in lettuce. MSc thesis, McGill University, Montreal, QC, Canada. 77 pages.

Van der Heyden, H., Carisse, O., and Brodeur, L. 2012. Comparison of monitoring based indicators for initiating fungicide spray programs to control Botrytis leaf blight of onion. Crop Protect. 33:21-28.

Wu, B. M., Subbarao, K. V., and van Bruggen, A. H. C. 2000. Factors affecting the survival of Bremia lactucae sporangia deposited on lettuce leaves. Phytopathology 90:827-833.

Wu, B. M., van Bruggen, A. H. C., Subbarao, K. V., and Pennings, G. G. H. 2001. Spatial analysis of lettuce downy mildew using geostatistics and geographic information systems. Phytopathology 91:134-142.

Wu, B. M., van Bruggen, A. H. C., Subbarao, K. V., and Scherm, H. 2002. Incorporation of temperature and solar radiation thresholds to modify a lettuce downy mildew warning system. Phytopathology 92:631-636.

Wu, B. M., van Bruggen, A. H. C., Subbarao, K. V., and Scherm, H. 2002. Incorporation of temperature and solar radiation thresholds to modify a lettuce downy mildew warning system. Phytopathology 92:631-636.

CHAPITRE 4

EFFICIENCE CONTAMINATRICE DE QUATRE LIGNÉES CLONALES DE *PHYTOPHTHORA INFESTANS* ET LA QUANTIFICATION DES SPORANGES BASÉE SUR L'ADN

Résumé

Le risque potentiel de développement du mildiou de la pomme de terre et les pertes subséquentes de rendement sont liés en partie à la dispersion aérienne des sporanges de *Phytophthora infestans*. Des études récentes suggèrent qu'un réseau de capteurs de spores peut être une approche appropriée pour la détection hâtive de l'inoculum provenant de l'extérieur du site de production. Cependant, pour rendre un tel réseau plus fiable comme outil de gestion et faciliter son adoption, l'information sur l'efficience contaminatrice et un outil moléculaire pour compter les sporanges sont nécessaires. Des expériences ont été ainsi réalisées dans une chambre de croissance pour étudier l'efficience contaminatrice de quatre lignées clonales de *P. infestans* (US-8, US-11, US-23, et US-24) en mesurant la concentration aérienne de sporanges et l'intensité de la maladie qui en résulte. La relation entre la concentration aérienne de sporanges et le nombre de lésions par feuille était exponentielle. Pour une même concentration, les sporanges de US-23 causent un nombre de lésions significativement plus élevé que les sporanges des autres lignées clonales. Dans des conditions optimales, une concentration aérienne de 10 sporanges m^{-3} de US-23 était suffisante pour provoquer une lésion par feuille, alors que pour les autres lignées clonales, il a fallu 15 à 25 sporanges m^{-3} pour atteindre la même intensité de maladie. De plus, un outil moléculaire sensible a été développé pour quantifier les sporanges de *P. infestans*, cet outil présente une sensibilité de détection d'un sporange.

La portée de l'article et les contributions respectives des auteurs

Cet article est la suite logique de l'article publié dans Plant Pathology. Cette étude a permis d'établir le lien entre la concentration aérienne de spores et l'intensité du mildiou de la pomme de terre (efficience contaminatrice). Aussi, elle permet d'expliquer en partie pourquoi la lignée clonale US-23 domine la population de *P. infestans* en Amérique du Nord. De plus, un outil moléculaire pour le comptage des spores a été développé dans l'optique de rendre l'implantation d'un réseau de capteurs de spores plus efficiente et de réduire les erreurs intrinsèques au comptage des spores au microscope.

La contribution des auteurs dans cet article est comme suit : j'ai effectué toutes les analyses statistiques et rédigé toutes les parties du manuscrit ainsi que les figures et tableaux pour la publication avec l'aide de M^{r} Tremblay. M^{r} Tremblay et D^{r} Couillard ont aidé à l'optimisation du test moléculaire. M^{me} Gobeil-Richard a effectué certains des tests de validation du marqueur moléculaire. D^{r} Lévesque et M^{me} Rocheleau ont élaboré les amorces du test. M^{r} van der Heyden, D^{r} Carisse, D^{r} Couillard et D^{r} Beaulieu ont agi comme conseillers et ont révisé l'article pour sa publication. Cette recherche a été sous la supervision de la D^{r} Carisse et D^{r} Beaulieu.

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INFECTION EFFICIENCY OF FOUR *PHYTOPHTHORA INFESTANS* CLONAL LINEAGES AND DNA-BASED QUANTIFICATION OF SPORANGIA

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4.1. Abstract

The presence and abundance of pathogen inoculum is with host resistance and environmental conditions a key factor in epidemic development. Therefore, several spore-sampling devices have been proposed to monitor pathogen inoculum above fields. However, to make spore sampling more reliable as a management tool and to facilitate its adoption, information on infection efficiency and molecular tools for estimating airborne sporangia concentration are needed. Experiments were thus undertaken in a growth chamber to study the infection efficiency of four clonal lineages of *P. infestans* (US-8, US-11, US-23, and US-24) by measuring the airborne sporangia concentration and resulting disease intensity. The relationship between the

airborne sporangia concentration and the number of lesions per leaf was exponential. For the same concentration, the sporangia of US-23 caused significantly more lesions than the sporangia of the other clonal lineages did. Under optimal conditions, an airborne sporangia concentration of 10 sporangia m^{-3} for US-23 was sufficient to cause one lesion per leaf, whereas for the other clonal lineages, it took 15 to 25 sporangia m^{-3} to reach the same disease intensity. However, in terms of diseased leaf area, there was no difference between clonal lineages US-8, US-23 and US-24. Also, a sensitive quantitative real-time polymerase chain reaction (qPCR) tool was developed to quantify *P. infestans* airborne sporangia with detection sensitivity of one sporangium. The specificity of the qPCR assay was rigorously tested for airborne inoculum and was either similar to, or an improvement on, other published PCR assays. This assay allows rapid and reliable detection and quantification of *P. infestans* airborne sporangia and thereby, facilitates the implementation of spores-sampling network.

4.2. Introduction

Potato late blight epidemics have become increasingly problematic in many parts of the world [1–3]. *Phytophthora infestans*, the causal agent of late blight disease in solanaceous plants, has been under investigation for the past 150 years. The disease spreads very quickly under cool, humid conditions when sporangia are produced on infected leaves and further splashed or wind-blown onto nearby plants [2]. Worldwide losses caused by late blight have been estimated at billions of U.S. dollars each year [3]. Management strategies for late blight are based essentially on the timing and efficiency of fungicide applications. Thus, most fungicide applications are timed using decision-support systems (DSSs), which target the optimal moment for fungicide application [4, 5].

Several DSSs that forecast potato late blight have been developed to guide decision making about fungicide applications in different potato production areas around the world [6]. These systems, which are used for predicting the risk of infection, are considered useful tools for controlling late blight with fewer applications of chemical fungicides than fixed-interval fungicide spray programs [6,7]. However, the major constraint of DSSs is the absence of information on the presence and the quantity of airborne inoculum in potato fields. Consequently, DSSs can overestimate or underestimate late blight risk [8]. Recent studies suggested that including the information from monitoring of *P. infestans* airborne inoculum could improve DSSs [5, 8].Those studies suggested that combining a spore-sampling network with a DSS could potentially aid targeting the optimal time to apply a disease-control product [5].

The potential consequences of late blight infection force growers to frequently spray their crops from the time the plants meet between rows all the way until harvest. This situation has been addressed by combining management strategies such as using resistant cultivars, altering planting practices, using certified seed, using DSSs, measuring airborne inoculum etc. [5, 8, 9]. However, to rationalize fungicide use and minimize the build-up of fungicide resistance, these measures should be applied in a coordinated and integrated manner. For example, airborne sporangia can be a counterweight to the DSS risk estimate: a high risk combined with a significant airborne sporangia concentration (ASC) will trigger fungicide spraying [8]. Therefore, the link between ASC and late blight intensity, along with the variation in disease intensity between different clonal lineages of *P. infestans*, should be studied. In North America, the sexual reproduction of *P. infestans* is limited, and thus the cycle of aerial asexual sporangia dispersal plays a crucial role in late blight development [10, 11]. Also, increases in the intensity of potato late blight in North America coincide with major genetic changes in the *P. infestans* population [12]: in 2009, for example, many producers lost practically all of their crops mostly because of the rapid spread of new clonal lineage of *P. infestans* and fungicide resistance [1]. Because they seem

to present different epidemiological characteristics [10, 12], clonal lineages are expected to be distinctive in terms of infection efficiency. In this context, infection efficiency is defined as the ratio of late blight intensity (number of lesions per leaf or disease severity) to ASC. Moreover, to improve the reliability of the information derived from measurements of airborne inoculum, a reliable quantitative tool is needed to accurately estimate inoculum concentrations.

The conventional method for estimation of *P. infestans* airborne sporangia is based on counting under microscope, which is time consuming, especially in the context of field sampling for rapid decision making. Instead methods for molecular quantification of *P. infestans* airborne inoculum could be developed. The sequence of the *P. infestans* genome is now complete and available [13], and thus numerous molecular tools have been developed [14–15] for use not only to understand the mechanisms that regulate the organism's survival and resistance responses or pathogenesis but also to conduct genotyping and phenotyping studies [16]. Over the past 20 years, several molecular tools for detecting or quantifying *Phytophthora* propagules on leaves and soil have been developed and used to improve late blight management [15–17, 18–19].

The disease triangle is a fundamental and unique principle describing factors involved in plant disease causation. Indeed, plant disease is prevented upon elimination of any one of the three components of disease triangle, which are, a susceptible host, a pathogen, and a favourable environment (e.g. weather conditions) [20]. Considerable efforts have been made to identify or develop late blight-resistant varieties and reliable weather forecasting models. However, in integrated manner presence and abundance of pathogen inoculum is at least as important as host resistant and environmental conditions. Over the past 25 years, several spore-sampling devices have been proposed to monitor the pathogen inoculum above

fields. Therefore, spore-sampling networks have been successfully implemented at a regional scale in different production area around the world [5–8,21,22]. For example, farmers in different potato production area in Canada are using information derived from spore-sampling network as functional strategy to control late blight epidemics [8]. Similar network were successfully implemented in organic soil in southwest of Montreal to manage leaf blight of onion and lettuce downy mildew [8, 20]. Also, In Michigan, field advisers monitor airborne *Venturia inaequalis* ascospores in apple orchards with spore samplers and use automated information lines, fax networks, and e-mail to keep producers informed [8]. Nevertheless, in order to facilitate interpretation of information derived from spore-sampling for decision-making, further study should be done to determine the link between the airborne spore concentration and disease intensity (action threshold), to develop a molecular tool for quantification of airborne spore, and to determine the number of samplers required depending on the size of the production area. This situation led to the hypothesis that there is a link between ASC and late blight intensity. Hence, the specifics objectives of this study were (i) to determine the infection efficiency of four clonal lineages of *P. infestans* and compare late blight intensity among them and (ii) to develop a TaqMan qPCR assay for the detection and quantification of airborne sporangia of *P. infestans*. This study shows an exponential relationship between *P. infestans* airborne sporangia concentration and the number of lesions per potato leaf. As expected, the infection efficiency varies among *P. infestans* clonal lineages. Also, a sensitive quantitative polymerase chain reaction (qPCR) assay that can detect low concentrations of *P. infestans* sporangia in air samples was developed. These results are expected to improve and facilitate interpretation of information derived from the spore-sampling network.

4.3. Materials and Methods

Agriculture and AgriFood Canada (AAFC) provided the experimental infrastructure for each activity in this study and no specific permissions were required for these activities. Also, the study did not involve endangered or protected species. All data are available in Tables, Figures and Supporting Information files.

4.3.1. Data collection to determine the infection efficiency of *P. infestans* clonal lineages

Experimental design. Unconventional assay was designed in a growth chamber to investigate the relationship between airborne inoculum and late blight intensity. A proof of concept of this essay was done in a previous study [22]. A total of 20 potato plants (cv. Russet Burbank) that had been produced in a greenhouse and had reached the 10-to-13-leaf stage were placed 0.15 m apart on the bottom of a growth chamber (PGC20 growth chamber; Conviron, Winnipeg, MB, Canada). In the upper part of the chamber, 4-to-8 potato leaves that had sporulating lesions on them and that had been produced from inoculated plants maintained in the growth chamber were placed 1.20 m above the potato plants so that the plants would be infected by airborne sporangia in a manner similar to infection under field conditions. To promote infection, the conditions in the growth chamber were 15 h of darkness, 9 h of daylight, 18°C, 100% relative humidity, and a leaf-wetness period of 6 h. While the sporulating lesions were in the growth chamber, a rotating-arm spore sampler (Compagnie de recherche Phytodata inc., Sherrington, QC, Canada) placed 0.5 m above the potato plant canopy was used to monitor sporangia concentrations. The sampler ran 30% of time during the 15 h of darkness for a total of 4 h (alternating between 4 min on and 11 min off), and the effective air-sampling rate was 21.65 L/min. After 14 h, the

sporulating leaves were removed, and the growth chamber was maintained at 18°C and 90% relative humidity for 6 d in a cycle of 9 h of darkness and 15 h of daylight. The ASCs were also monitored to make sure that no airborne sporangia remained in the growth chamber one hour after the sporulating leaves had been removed. Sporangia caught on the sampling surfaces (rods) were counted within 24 h after sampling with a microscope at 250× magnification. The number of sporangia per rod was converted to sporangia per cubic meter of air as follows: (number of sporangia per rod × 1,000 L/m^3/h)/(21.65 L/min per rod × 60 min/h × 4 h). This experiment was repeated 14, 16, 14, and 17 times for clonal lineages US-24, US-23, US-11, and US-8, respectively. To obtain a range of airborne sporangia concentration, the number of sporulating lesions that were introduced in the growth chamber was different in each assay. Airborne sporangia concentrations ranging between 0 (no sporulating lesions) and 40 sporangia m−3 were measured for each of the clonal lineages. The control for each lineage was the first experimental run without sporulating lesions in the growth chamber (control). To evaluate the uniformity of sporangial distribution in the growth chamber, sporangia collected on microscope slides placed in the bottom of the growth chambers were counted and compared.

All clonal lineages were collected in Canada, the clonal lineages US-23, US-24 and US-8 were kindly provided by Dr. Khalil Al-Mughrabi (New Brunswick Department of Agriculture, Aquaculture and Fisheries) and US-11 was provided by Dr. Katherine Dobinson (Agriculture and Agri-Food Canada, southern crop protection and food research center). For each clonal lineage, a mixture of three isolates was used to infect potato plants and produce sporulating lesions.

Measurement of disease. Seven day after each inoculation, the number of lesions per leaf and disease severity were assessed on all trap plants by randomly selecting five leaves on each plant. Disease severity was recorded by visual estimation of the

leaf area with late blight lesions. Severity was estimated on a scale from 1 to 10, where: 1 = no lesion; 2 = 0+__ 3%; 3 = 3+__ 5%; 4 = 5+__10%; 5 = 10+__25%; 6 = 25+__50%; 7 = 50+__75%; 8 = 75+__85%; 9 = 85+__95% and 10 = 95+__100% of leaf area with late blight symptoms [23, 24]. The superscript (+) means value just slightly above the indicated value.

3.3.1.1. Data analysis

Before analysis, the disease severity rating was converted to the severity percentage using the interval midpoint [22], as follows: 1 = 0%, 2 = 1.5%, 3 = 4%, 4 = 7.5%, 5 = 17.5%, 6 = 37.5%, 7 = 62.5%, 8 = 80%, 9 = 90%, and 10 = 97.5%. The ASCs were grouped into the following three categories: low for ASC ≤ 5 sporangia m^{-3}, medium for 5 < ASC ≤ 10 sporangia m^{-3}, and high for ASC > 10 sporangia m^{-3}. First, normality of the data was tested using the Shapiro–Wilk test, and then, if necessary, logarithmic transformation was done to improve normality. All statistical tests were performed in R software (version 3.0.0; R Foundation for Statistical Computing, Vienna, Austria).

Infection efficiency of *P. infestans* clonal lineages. A scatter plot representing the number of lesions per leaf as a function of ASC was prepared. To describe the relationship between the number of lesions per leaf and ASC, a two-parameter exponential rise to a maximum function was fitted to the data, as follows:

$$y = a(1 - \exp(-bx)) \tag{1}$$

Where y is the number of lesions per leaf, x is the ASC, a is the asymptote, and $\frac{1}{b}$ is the rate. The model's appropriateness and goodness of fit to the data were assessed using an adjusted R^2 and a concordance correlation coefficient (ρ_c) [22].

A scatter plot representing the severity (percentage of leaf area diseased) as a function of ASC was prepared. To describe the relationship between those two variables, a linear function with an ordinate intercept of zero was fitted to the data. Two factors were considered, ASC and clonal lineage. ASC is a factor with three levels: low, medium and high. Clonal lineage is a factor with four levels: US-8, US-11, US-23 and US-24. A factorial analysis of variance (ANOVA) was used to investigate statistical interactions, in which the number of lesions caused by clonal lineages depends on the level of ASC. Because the sample size for each level differed (unbalanced design), the factors were entered in different orders to see how much of a difference this makes to the outcomes of the analysis. The agricolae package in R (agricolae: Statistical Procedures for Agricultural Research, version 1.1-4, in statistical software R3.0.0 GUI 1.60; Lima, Peru) was used [23]. When the full model was statistically significant ($\alpha = 0.05$), means across the number of lesions per leaf among clonal lineages were compared, using the Tukey HSD (honest significant difference) post hoc comparison. A pairwise *t*-test was used to calculate the adjusted *P*-values (agricolae: Statistical Procedures for Agricultural Research) [23].

4.3.2. Development of the *P. infestans* real-time qPCR assay

4.3.2.1. Design of primers

Multiple sequence alignments were built from sequences originating from internal transcribed spacer (ITS) DNA segments of different oomycetes [24]. A short region with high dissimilarities among the *Phytophthora* species was identified within the ITS2 region. This DNA segment was submitted to the PrimerQuest tool (http://www.idtdna.com/Primerquest/Home/Index). The newly designed primers and the TaqMan probe set ITS2HR-pinf-F, ITS2HR-pinf-R, and ITS2HR-pinf-P amplified a 108-base-pair (bp) product and are listed in Table 4.1.

4.3.2.2. Exogenous internal positive control

To detect partial or complete inhibition of the qPCR reaction, as well as potential experimental errors, an exogenous internal positive control (EIPC) was developed and added in the DNA extraction and dilution solutions. The EIPC fragment (Table S1) consisted of double stranded DNA genomic blocks (gBlocks; Integrated D NA Technologies, Inc., Coralville, IA, USA) designed from a random DNA sequence o f 500 bp generated from Stothard P (2000) (http://www.bioinformatics.org/sms2/refere nce.html). Primers EIPC100-F and EIPC100-R and TaqMan probe EIPC100-P (Table 4.1) were designed from the EIPC sequence using Beacon Designer 8.13 (Premier Biosoft, Palo Alto, CA, USA) and generated a 100-bp amplicon.

Table 4.1. Primer and probe names and sequences for qPCR reactions for quantification of Phytophthora infestans sporangia

Primer/probe/sequence name	5′-3′ sequence
ITS2HR-pinf-F	TGG ACT GGT GAA CCA TGG CTC TT
ITS2HR-pinf-P[a]	TTG CGA AGT AGA GTG GCG GCT TCG GCT GC
ITSHR-pinf-R	CAA CAT TTC CCA AAT GGA TCG ACC CT
PITSstdF	AACTAGATAGCAACTTTC
PITSstdR	GTTTTCAGGTACTCTTTA
EIPC100-F	AGGCTAGCTAGGACCGATCAATAGG
EIPC100-P[b]	CCTATGCGTTCCGAGGTGACGACCTTGCC
EIPC100-R	AGTGCTTCGTTACGAAAGTGACCTTA

[a] Zen double-quenched fluorescent-labelled probe 5′-6-Fam, 3′-IBHQ1.
[b] Zen double-quenched fluorescent-labelled probe 5′-HEX, 3′-IBHQ1.

4.3.2.3. Preparation of DNA solution for construction of *P. infestans* ITS2 copy standard curve

DNA from *P. infestans* isolates (US-6, US-8, US-11, US-22, US-23, and US-24) was used for the PCR amplification of a 1004-bp ITS2 fragment. In a 50-µL reaction mixture containing 6 ng of genomic DNA, 1X Taq PCR buffer, 4.0 mM of $MgCl^{2+}$, 0.03 U/µL of SurePRIME Taq polymerase (MP Biomedicals, Solon, OH, USA), 0.4 mM of dNTPs (New England Biolabs Inc., Ipswich, MA, USA), and 200 nM each of primers PITSstdF and PITSstdR (Table 4. 1), the following PCR conditions were used: 95°C for 15 min, followed by 40 cycles at 94°C for 15 s, 58°C for 45 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR fragments were purified using the Nucleospin PCR clean-up kit (Macherey-Nagel GmbH & Co., Düren, Germany) according to the manufacturer's recommendations. The purified products were quantified using a 2100 Bioanalyzer instrument (Agilent Technologies,

Santa Clara, CA, USA). The DNA was analyzed according to the manufacturer's instructions and converted into copy numbers, calculated as copy number = [(concentration of amplicon in g/µL)/(1004 bp × 660 g/mole) × (6.022×10^{23})]. This DNA was then used for optimizing the TaqMan qPCR assay and generating the ITS2 copy number standard curve.

4.3.2.4. Preparation of DNA solution for construction of *P. infestans* sporangia standard curve

To prepare the sporangial standard curve, dry sporangia of US-8 and US-11 were harvested from sporulating potato leaves. The sporangia were harvested from four to six fresh sporulating lesions, avoiding the necrotic area, using a truncated disposable 10-mL pipette connected to a vacuum pump. Approximately 5 mL of isopropanol 100% (Sigma-Aldrich Canada Ltd.) was then poured into the pipette to suspend the harvested sporangia. Suspensions of 3125 sporangia were transferred into 2-mL screw-cap tubes containing 100 mg of 425-to-600-µm glass beads (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). The isopropanol was then evaporated using a Vacufuge (Eppendorf Canada Ltd., Mississauga, Ontario, Canada) at 60°C for 20 min and then the tubes were stored at −20°C until DNA extraction. To maximize the quantity of DNA pulled out, a lossless DNA extraction method without purification was used. Lysis of sporangia and DNA recovery were accomplished in a single extraction tube containing a silicon-coated spore trap rod and 75 µl of ACS reagent-grade petroleum ether (Sigma-Aldrich). A silicon coated polystyrene spore trap rod was added to the tubes to mimic same lysis conditions that field collected samples as discussed later in this manuscript. The tubes were shaken for 20 s at 4 m/s using a Fast-Prep instrument (MP Biomedicals, Solon, Ohio) and then centrifuged for 5 s at 10,000 x g to pellet the sporangial debris at the bottom of the tubes. Petroleum ether was evaporated using the Vacufuge instrument for 20 min at 60°C.

The sporangial lysate was suspended in 300 µL of DNA extraction solution consisting of nuclease-free water, salmon sperm DNA at 10 ng/µL (Life Technologies Inc., Burlington, ON, Canada), 5 % Chelex 100 (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), 100 ng/µL bovine serum albumin (New England Biolabs Inc.), and an EIPC fragment at 2×10^2 copies/µL. To complete DNA extraction, the tubes were then heated at 105°C for 20 min in a dry bath, briefly agitated using a vortex, and centrifuged at 4°C and 15,000 × *g*. The supernatants were kept at 4°C for a maximum of 1 h to avoid DNA degradation. The DNA was then used for optimizing the TaqMan qPCR assay and constructing the *P. infestans* sporangia standard curve.

4.3.2.5. Construction of standard curves to estimate the number of spores from the number of ITS2 copies

The ITS2 copy standard curve was constructed by 10-fold serial dilutions of the 1004 bp *P. infestans* ITS-2 gene fragment, ranging from 1×10^5 to 1 copy/µL in a DNA dilution solution consisting of nuclease-free water containing salmon sperm DNA at 10 ng/µL and EIPC at 2×10^2 copies/µL. The sporangia standard curve was constructed by five-fold serial dilutions of 3125 to one *P. infestans* sporangium in the DNA dilution solution as described above. For both curves, the procedure was repeated three times. A three-step regression analysis was used to determine the number of copies of ITS2 per sporangia. First, regression analysis was used to establish the relationship between the quantification cycle threshold (Cq) value and the logarithm of the number of ITS2 copies. Second, regression analysis was used to establish the relationship between the Cq value and the logarithm of the number of sporangia. Finally, the predicted number of sporangia was regressed against the predicted number of DNA fragments for Cq values of 18 to 30. The resulting equation was used to determine the number of sporangia from the number of copies obtained from the qPCR assay. The regression procedure (PROC REG) of SAS (version 9.1;

SAS Institute Inc., Cary, NC, USA) was used to estimate the intercept and slope regression parameters.

4.3.2.6. Extraction procedure of *P.infestans* DNA from field sampler

Phytophthora infestans DNA from field collected spore trap polystyrene rods were extracted using lossless DNA extraction as described previously. However, during the first step of the procedure, single polystyrene rods were placed directly in each extraction tube with 75 µl of petroleum ether instead of suspending sporangia in isopropanol. Supernatants were then maintained at 4°C for a maximum of 1 h and used as the DNA solution for qPCR sporangia assessment.

4.3.2.7. Evaluation of the TaqMan qPCR assay procedure

A standard curve of the *P. infestans* 1004-bp *P. infestans* fragment was established as described above in the DNA dilution solution. The reactions were performed in a 25 µL final volume containing 5 µL of DNA extract, 12.5 µL of 2x QuantiFast Multiplex Probe PCR Master Mix (Qiagen Inc, Mississauga, ON, Canada), 300 nM each of primers ITS2HR-pinf-F, ITS2HR-pinf-R, EIPC100-F and EIPC100-R and 200 nM each of TaqMan probes ITS2HR-pinf-P and EIPC100-P. The two-step PCR conditions were 95°C for 5 min, followed by 40 cycles at 95°C for 30 s and 62°C for 30 s in a Mx3005P qPCR Instrument (Agilent Technologies, Santa Clara, CA, USA). The results were evaluated for the efficiency of *P. infestans* amplification and the stability of EIPC detection among the dilutions.

4.3.2.8. Specificity of the qPCR assay

The specificity of the TaqMan qPCR assay was tested on several species of *Phytophthora* as well as on *Pythium vexans* (Table 4.2). The TaqMan qPCR assay was performed as described previously using 100 pg of genomic DNA added to the 25-µL assay reaction. The qPCR reaction was performed using the QuantiFast Multiplex PCR +R Kit (Qiagen Inc.). The two-step qPCR conditions were the same as those described above.

4.3.3. Laboratory validation of the qPCR assay

Drops of a *P. infestans* sporangia solution in isopropanol 100% (as described above) were deposited onto glass rods coated with silicon vacuum grease [25]. The rods were kept at room temperature for about 5 min to allow the isopropanol to evaporate. The number of spores per rod was counted under a light microscope at 250× magnification. DNA was extracted as described above, and the number of spores was estimated with the qPCR assay and standard curve as described above. The experiment was repeated 23 times. Regression analysis was used to establish the relationship between the number of sporangia estimated by qPCR and the number estimated by microscopy. The analysis was performed using the regression procedure.

4.4. Results

4.4.1. Relationship between airborne sporangia concentration and late blight intensity

The relationship between ASC and the number of lesions per leaf is shown in Figure 4.1. When the potato plants were exposed to a range of ASCs (between 0 and 40 sporangia m^{-3}) for a 6-h leaf-wetness period, the subsequent number of lesions per leaf increased exponentially as the ASC increased. The maximum numbers of lesions that US-8, US-11, US-23, and US-24 could cause were 2.27, 1.40, 4.81, and 2.02, respectively. When the potato plants were exposed to a range of ASCs between 0 and 10 sporangia m^{-3} (low and medium ASCs), the number of lesions per leaf was not significantly different between clonal lineages (Tables 4.2 and 4.3, Figure 4.2). However, when the ASC was above 10 sporangia m^{-3} (high ASC), the number of lesions caused by US-23 was significantly ($P = 0.01$, $P < 0.00$) higher than the number of lesions caused by the other clonal lineages (US-8, US-11, and US-24) (Table 4.4, Figure 4.2). Also, US-8 and US-24 were not significantly different in terms of number of lesions, but US-8 caused significantly more lesions than US-11 did (Table 4.4, Figure 4.2). An ASC of 10 sporangia m^{-3} for US-23 caused one lesion per leaf, whereas for the other clonal lineages, it took 15 to 25 sporangia m^{-3} to cause one lesion per leaf (Figure 4.1). Clonal lineage US-23 showed higher aggressiveness in terms of the number of lesions produced than the other clonal lineages did (Figure 4.2). Also, the sporangia were uniformly distributed in the growth chamber. A two-parameter exponential rise to a maximum was fitted to the data. The clonal lineages tested in the study were US-8, US-11, US-23, and US-24.

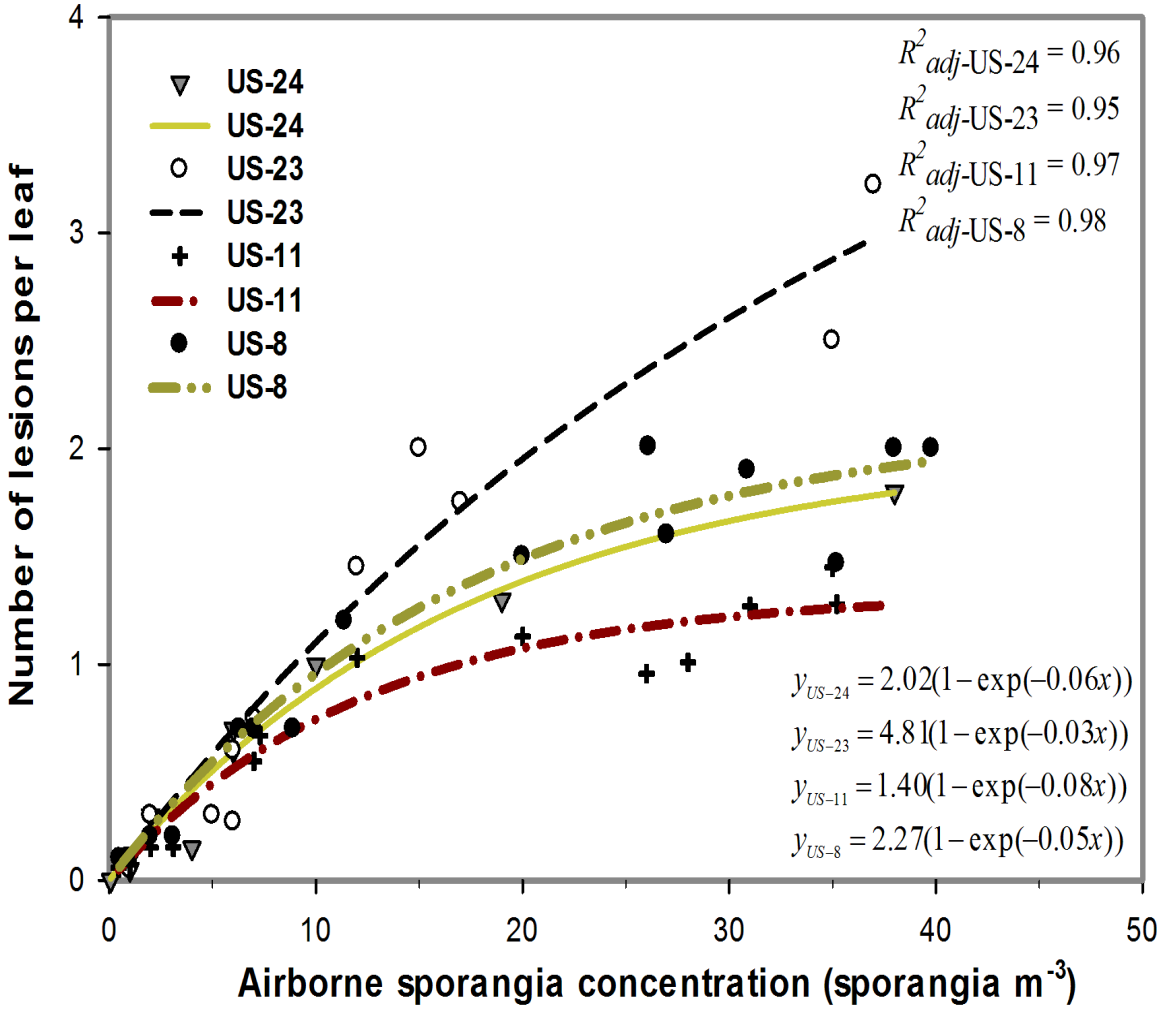

Figure 4.1. The number of lesions per leaf as a function of airborne sporangia concentration for four *Phytophthora infestans* clonal lineages.

Table 4.2. Isolates/DNA of species used in this investigation and evaluation of species specificity of the TaqMan assay. Fluorescence is indicated as detected (+) or undetected (−) for *Phytophthora* species.

Isolate/DNA	Species	Host	Origin	PCR reaction	Provided by
Pi 09-30-DO1	*P. infestans* (US-6)	Solanum tuberosum	Prince Edward Island, Canada	+	1
Pi 09-09-A03	*P. infestans* (US-8)	Solanum tuberosum	Prince Edward Island, Canada	+	1
Pi 281-P3C10	*P. infestans* (US-8)	Solanum tuberosum	Prince Edward Island, Canada	+	1
Pi 31-CO1	*P. infestans* (US-11)	Solanum tuberosum	Prince Edward Island, Canada	+	1
Pi-Rusinek	*P. infestans* (US-22)	Solanum tuberosum	Ithaca, New York, USA	+	2
Pi-09-225-P3B10	*P. infestans* (US-8)	Solanum tuberosum	Ithaca, New York, USA		2
Pi-US23NB	*P. infestans* (US-23)	Solanum tuberosum	New Brunswick, Canada	+	3
Pi-US24NB	*P. infestans* (US-24)	Solanum tuberosum	New Brunswick, Canada	+	3
Pi-3-1-11	*P. infestans* (US-24)	Solanum tuberosum	Manitoba, Canada	+	4
Pi-4-1-11	*P. infestans* (US-24)	Solanum tuberosum	Manitoba, Canada	+	4
P12 (P1B10)	*P. capsici*	N/A	Ithaca, New York, USA	−	2
H7 (P2B10)	*P. capsici*	N/A	Ithaca, New York, USA	−	2
P13365	*P. andina*	Solanum brevifolium	Ecuador	+	5
P3008	*P. mirabilis*	Mirabilis jalapa	Mexico	+	5
P6609	*P. phaseoli*	Phaseolus lunatus	Maryland, USA	+	5

Isolate/DNA	Species	Host	Origin	PCR reaction	Provided by
N/A	*P. megasperma*	Glycine spp.	N/A	−	5
P1699	*P. erythroseptica*	Solanum tuberosum	Ohio, USA	−	5
P1435	*P. fragariae*	Fragaria × ananassa	England	−	5
N/A	*P. pseudotsugae*	N/A	Maryland, USA	−	5
P0991	*P. nicotianae*	Citrus spp.	California, USA	−	5
N/A	*P. ideai*	Rubus occidentalis	California, USA	−	5
P10303	*P. ramorum*	Rhododendron spp.	Netherlands	−	5
P6497	*P. sojae*	Glycine max	Mississippi, USA	−	5
P0767	*P. citricola*	Syringa spp.	Canada	−	5
N/A	*P. clandestine*	N/A	Maryland, USA	−	5
P3281	*P. inundata*	Rubus idaeus	New York, USA	N/A	5
P7889	*P. medicagae*	Trifolium repens L.	New York, USA	N/A	5
N/A	*P. iranica*	Myrtus nivellei	Maryland, USA	−	5
P2021	*P. cinnamomi*	Camellia japonica	California, USA	−	5
N/A	*P. tentaculata*	Mimulus aurantiacus	Maryland, USA	−	5
P1703	*P. crypthogea*	Solanum tuberosum	Ohio, USA	N/A	5
P3273	*P. brassicae*	Brassica oleracea	Netherlands	−	5
P10190	*P. meadii*	Citrus spp.	India	N/A	5
N/A	*P. inflate*	Ulmus spp.	Maryland, USA	−	5
N/A	*P. psychrophila*	N/A	Maryland, USA	−	5
P15550	*P. quercina*	Quercus rubra	Minnesota, USA	−	5
P10695	*P. insolita*	Asparagus officinalis	California, USA	N/A	5
N/A	*P. palmivora*	Cocos nucifera	Maryland, USA	N/A	5
P15553	*P. europaea*	Quercus alba	West Virginia, USA	−	5
N/A	*P. arecae*	Citrus spp.	Maryland, USA	−	5
P0592	*P. cambivora*	Abies procera	Oregon, USA	N/A	5

Isolate/DNA	Species	Host	Origin	PCR reaction	Provided by
P3849	*P. drechsleri*	Actinidia deliciosa	California, USA	N/A	5
A1000	*P. heveae*	Rhododendron spp.	North Carolina, USA	N/A	5
P3939	*P. ilicis*	Ilex spp.	British Columbia, Canada	N/A	5
N/A	*Pythium vexans*	Citrus spp.	Maryland, USA	−	5
P1462	*P. trifolii*	Trifolium vesiculosum	Mississippi, USA	N/A	5
P7293	*P. citrophthora*	Rhododendron sp	Massachusetts, USA	−	5
P11061	*P. hedraiandra*	Viburnum tinus	Balearic Islands, Spain	−	5
P6137	*P. gonapododyides*	Pseudotsuga menziesii	Oregon, USA	N/A	5
P6404	*P. rubi*	Rubus idaeus	West Germany	N/A	5
P0647	*P. hibernalis*	Citrus sinensis	California, USA	N/A	5
P10971	*P. foliorum*	Rhododendron spp.	California, USA	N/A	5
P16355	*P. pseudosyringae*	Umbellularia californica	California, USA	−	5
P1372	*P. katsurae*	Cocos nucifera	Hawaii, USA	−	5
P. lateralis	*P. lateralis*	N/A	N/A	N/A	6
P. syringae	*P. syringae*	N/A	N/A	−	6

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Table 4.3. Factorial analysis of variance of the number of lesions per leaf among clonal lineages of *Phytophthora infestans* in response to the airborne sporangia concentration in the growth chamber

Source	Degrees freedom	Mean square	P-values
Clonal lineages	3	0.502	0.001 **
ASC	2	13.195	< 2e-16 ***
ASC:Pathotype	6	0.420	0.00042 ***

Signif. codes: **, and *** represent $P < 0.01$, and $P < 0.001$, respectively

Table 4.4. Results of multiple comparisons of the number of lesions per leaf among clonal lineages of *Phytophthora infestans.*

ASC	Clonal lineage		Mean difference	P_{adj}	95% confidence	
					Lower bound	Upper bound
Low	US-8	US-11	−0.0033	1.00	0.6013	0.5947
		US-23	−0.0119	1.00	−0.5613	0.5375
		US-24	0.0166	1.00	0.5328	0.5661
	US-11	US-23	0.0085	1.00	−0.5697	0.5868
		US-24	−0.0200	1.00	−0.5983	0.5583
	US-23	US-24	0.0285	1.00	−0.5564	0.4993
Medium	US-8	US-11	0.0500	1.00	−0.8515	0.9515
		US-23	0.2000	0.99	−0.5543	0.9543
		US-24	0.3000	0.99	−0.6015	1.2015
	US-11	US-23	−0.1500	1.00	−1.0053	0.7053
		US-24	−0.2500	0.99	−1.2376	0.7376
	US-23	US-24	−0.1000	1.00	−0.9553	0.7553
High	US-8	US-11	0.5410	0.02*	0.0299	1.0522
		US-23	−0.4875	0.01*	−1.0505	0.0755
		US-24	0.5375	0.12	−0.0673	1.1423
	US-11	US-23	1.0285	<0.00***	0.4502	1.6068
		US-24	0.0035	1.00	−0.6154	0.6226
	US-23	US-24	−1.0250	<0.00***	−1.6875	−0.3624

Significance codes: *, and *** represent $P < 0.05$, and $P < 0.001$, respectively.
Note: The mean differences and 95% confidence levels were calculated using the Tukey HSD (honest significant difference) function. Pairwise t-test was used to calculate the adjusted P-values (P_{adj}) ($\alpha = 0.05$).
Airborne sporangia concentration level: ASC ≤ 5 sporangia m^{-3} is considered to be low, 5 < ASC ≤ 10 sporangia m^{-3} is considered to be medium, and ASC >10 sporangia m^{-3} is considered to be high.

The relationship between ASC and the percentage of leaf area diseased is shown in Figure 4.3. Late blight severity increased linearly as ASC increased. The slopes of linear regression were 1.5, 0.98, 1.6, and 1.6 for clonal lineages US-8, US-11, US-23, and US-24, respectively (Figure 4.3). For ASCs ranging between 10 and 40 sporangia m^{-3}, the proportion of leaf area diseased caused by US-8, US-23, and US-24 increased twofold when the ASC doubled. As shown in Figure 3, an ASC of 1 sporangium m^{-3} was sufficient to cause a severity of 1% for all clonal lineages. For US-8, US-23, and US-24, an ASC of 6 sporangia m^{-3} was sufficient to reach 10% leaf area diseased, whereas for US-11, 13 sporangia m^{-3} were required. Thus, US-11 showed lower aggressiveness in terms of severity than the other clonal lineages did.

4.4.2. Development of the *P. infestans* real-time qPCR assay

4.4.2.1. Estimation of the number of sporangia from the number of ITS2 gene copies and specificity of the TaqMan qPCR assay

The ITS copy standard curves generated by 10-fold serial dilutions of the 1004-bp amplicon were found to be linear, with efficiency of 103.10%, a regression coefficient of 0.99, a slope of 3.27, and an intercept of 35.93. Similarly, the sporangia standard curves generated by five-fold serial dilutions of sporangia DNA extractions were also linear, with efficiency of 102.70%, a regression coefficient of 0.99, a slope of 3.26, and an intercept of 29.51 (Figure 4.4). A third linear regression derived from the two previous regressions was used to determine the number of ITS copies per sporangium as follows: number of ITS copies per sporangia = (1/0.011) × fraction of DNA extraction added (Figure 4.4). In this equation, the intercept is 0 and the slope is 0.011. The qPCR assay cross-reacted with the closely related *Phytophthora* species *P. mirabilis*, *P. ipomoeae*, *P. andina*, and *P. phaseoli* (Table 4.2).

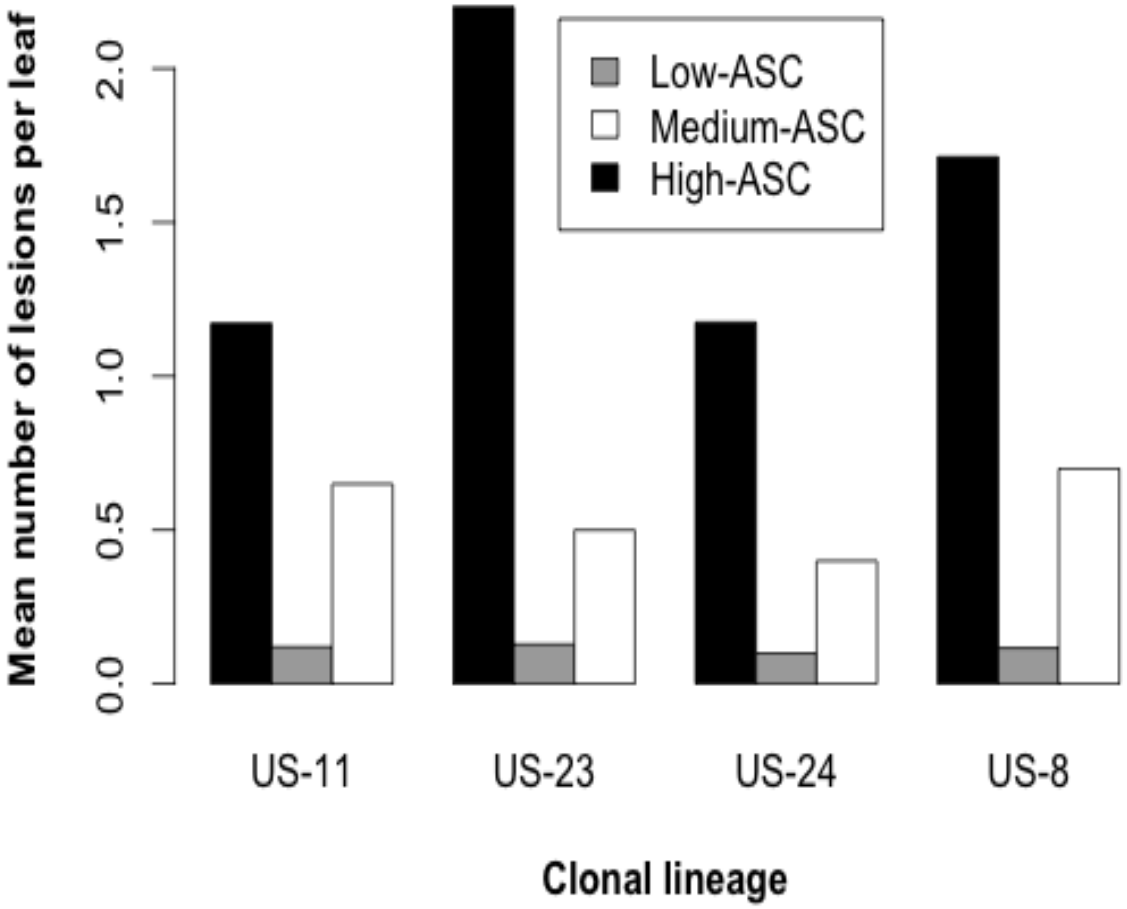

Figure 4. 2. The mean number of lesions per leaf of four *Phytophthora infestans* clonal lineages in high, medium and low level of airborne sporangia concentration (ASC). The clonal lineages tested in study were US-8, US-11, US-23, and US-24. Airborne sporangia concentration level (ASC) ≤ 5 sporangia m^{-3} is considered to be Low, 5 < ASC ≤ 10 sporangia m^{-3} is considered to be Medium, ASC >10 sporangia m^{-3} is considered to be High. The experiment was repeated 17 times for US-8, 14 times for US-11, 16 times for US-23 and 14 times for US-24.

4.4.3. Validation of the real-time qPCR assay

There is a linear relationship between the number of sporangia deposited onto the rods estimated with microscopy and the number of sporangia estimated with the TaqMan qPCR assay (R^2 = 0.99) (Figure 4.5). For all rods, no signals were detected from rods free of sporangia. TaqMan qPCR signals were obtained from all rods where at least one spore had been deposited.

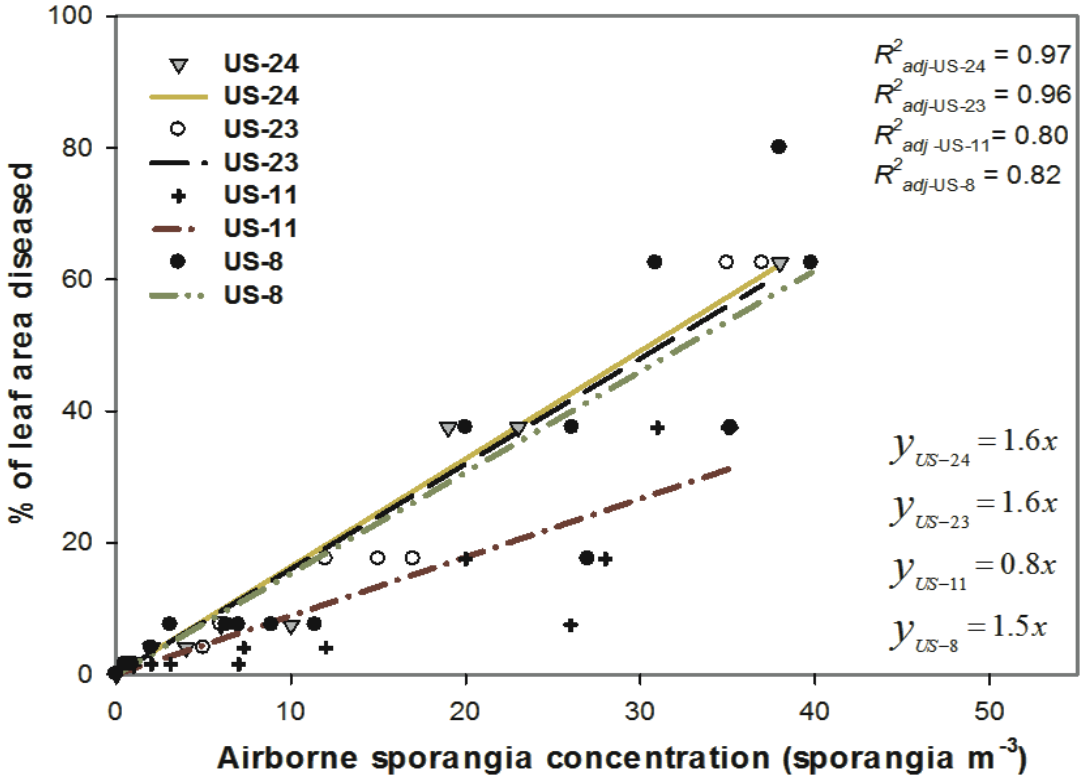

Figure 4.3. Relationship between the percentage of leaf area diseased and *Phytophthora infestans* airborne sporangia concentration. A linear function was fitted to the data. The clonal lineages tested in the study were US-8, US-11, US-23, and US-24.

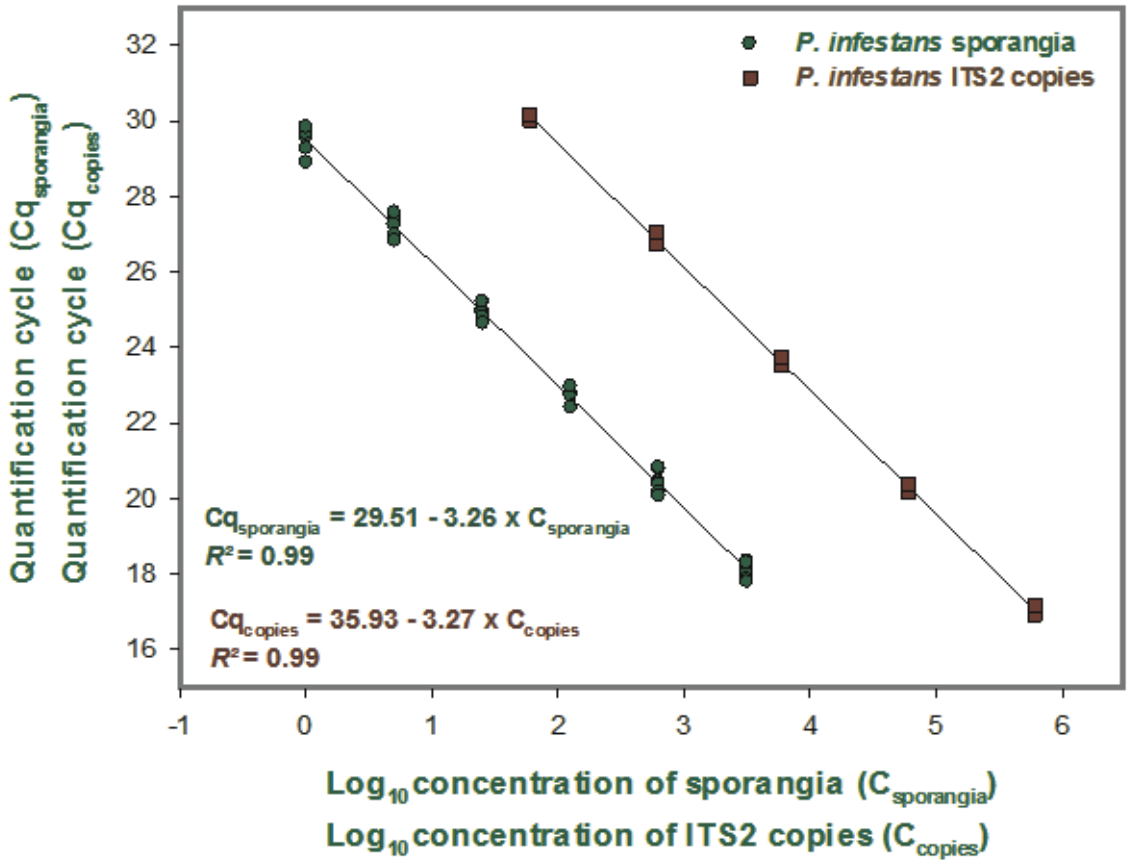

Figure 4. 4. Relationship between the quantification cycle (Cq) value and the log concentration of *Phytophthora infestans* sporangia and between the Cq value and the log concentration of internal transcribed spacer 2 (ITS2) copies.

4.5. Discussion

The presence of new clonal lineages in different regions of North America increases concern about late blight control, because new clonal lineages may have unique epidemiological characteristics [17]. Within a production season, no more than three clonal lineages are present and one of them will generally be predominant [19]. Therefore, information about which clonal lineage is present is needed once or twice

during the growing season. Models predicting disease development, such as decision support systems (DSSs) need to be adjusted to include differences in epidemiological characteristics among clonal lineages. Recent studies have suggested that, in supplement to the DSS, a sustainable potato late blight management system will benefit from quantification of the amount of inoculum by using spore-sampling devices [5, 8]. A high DSS risk estimated combined with significant airborne sporangia concentration (ASC) would trigger fungicide spraying [8]. However, the value of the information derived from spore samplers relies on the overall accuracy of the inoculum quantification and the determination of the infection efficiency. In this context, infection efficiency is defined as the ratio of the late blight intensity (number of lesions per leaf or disease severity) to the airborne sporangia concentration. Information on infection efficiency will help with understanding what level of ASC should be considered as an action threshold according to the epidemiological characteristics of each clonal lineage. In this study, the infection efficiency was investigated in a controlled environment, and a real-time qPCR assay for detecting and quantifying of *P. infestans* airborne inoculum was developed.

Under optimal infection conditions, potato late blight intensity increased as the airborne sporangia concentration (ASC) of *P. infestans* increased. The number of lesions per leaf increased exponentially as the ASC increased, and the maximum numbers of lesions that US-8, US-11, US-23, and US-24 could cause were 2.27, 1.40,4.81, and 2.02, respectively. Also, under high airborne sporangia concentration (above 10 sporangia m^{-3}) the number of lesions caused by US-23 was significantly higher than the number of lesions caused by the other clonal lineages. An ASC of 10 sporangia m^{-3} for US-23 was sufficient to cause one lesion per leaf whereas it took 15 to 25 sporangia m^{-3} for the other clonal lineages. Consequently US-23 showed more aggressiveness than did the other clonal lineages tested in this study. The rapidly spreading devastating epidemic of 2009 caused by US-23 and the fact that it is becoming the dominant clonal lineages in United States and Canada [17, 26, 27]

may be explained in part by that lineage's differences in terms of its capacity to produce a high number of lesions (infection efficiency). Indeed, US-8 has been the dominant clonal lineages for the past 15 years but it is gradually being replaced by US-23 [27]. Also, clonal lineages US-8 and US-24 were not significantly different in terms of infection efficiency. This result is supported by the findings of Danies et al. [17], which indicate that the pathogenicity characteristics of US-24 are similar to those of US-8. As well, the present results suggest that US-23 caused more lesions than clonal lineages US-8 and US-24 did, and US-23 was expected to cause greater diseased leaf area than US-8 and US-24 would cause.

Diseased leaf area increased linearly as the ASC increased. The slopes of linear regression were 1.5, 1.6 and 1.6 for clonal lineages US-8, US-23, and US-24, respectively. These results suggest that in terms of diseased leaf area, there was no difference between clonal lineages US-8, US-23 and US-24. Although this may seem a contradiction with the results described above, Danies et al. [17] found that US-8 and US-24 caused larger necrotic lesions on potato plants than US-23 did. It seems that US-23 caused more lesions, but US-8 and US-24 caused larger lesions, and thus the diseased leaf area was not different between US-8, US-23, and US-24. For those three clonal lineages, an ASC between 1 and 6 sporangia m^{-3} was sufficient to cause diseased leaf areas of 1% and 10%, respectively. These results can be useful for making late blight management decisions. Indeed, Stein and Kirk [4] suggested that delaying the initiation of any fungicide application until the estimated average foliar area with late blight reached 10% would result in disease development similar to an untreated control.

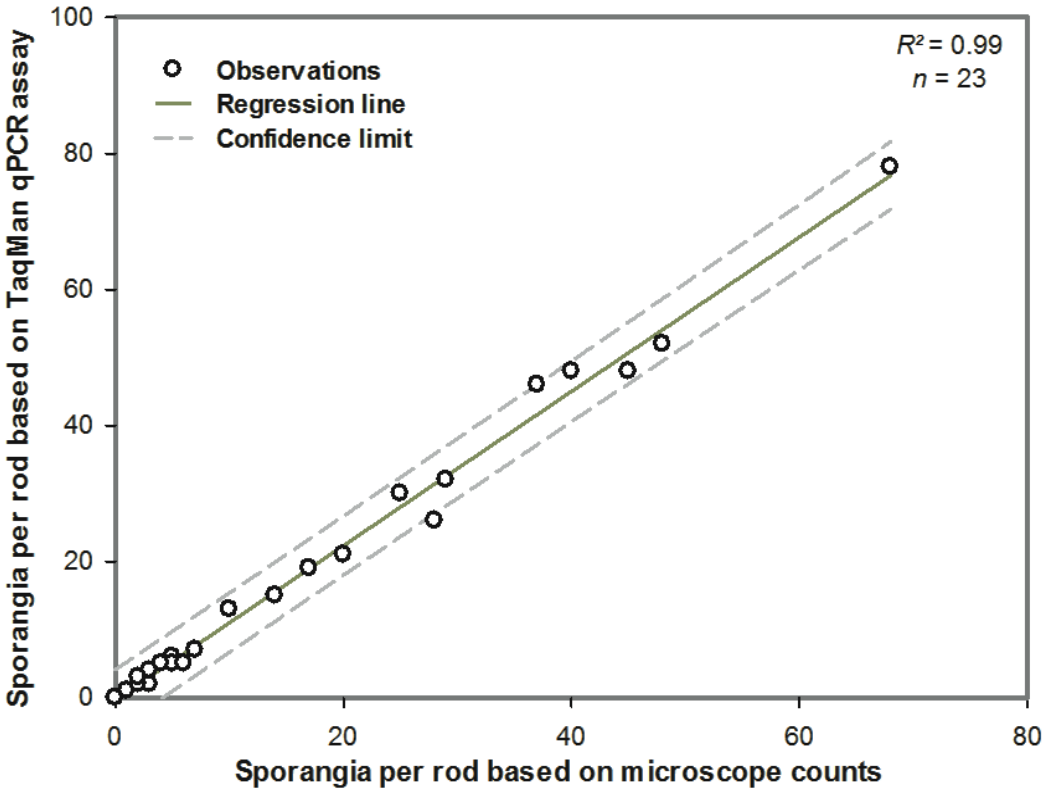

Figure 4.5. Relationship between estimates of the number of *Phytophthora infestans* sporangia deposited on silicon-greased rotating-arm sampler rods based on the *P. infestans* real-time qPCR assay and estimates based on microscope counts.

Also, most fungicides and fungicide mixtures reduce late blight development as long as they are applied before the diseased leaf area exceeds 1% [4]. However, the effects of temperature on whether sporangia release zoospores or instead of directly germinate may differentially influence the infection efficiency among clonal lineages [17]. Therefore, there is a need to study infection efficiency among clonal lineages in different ranges of temperature. The difference among clonal lineages could also potentially be attributed to differences in biotrophic growth that may have resulted in

an underestimation of the diseased leaf area due to difficulties defining the boundaries of lesions. Overall, the findings for infection efficiency point to a clear correlation between ASC and diseased leaf areas but this still needs to be validated under field conditions. It is also necessary to have a reliable ASC quantification tool other than a microscope, because microscopy is a complex and time-consuming process for field samples.

Because qPCR methods can overcome the drawbacks of counting and identifying by microscopy, quantitative PCR is a favoured tool for quantification, detection and identification of many organisms [25, 28]. To improve the detection and quantification of *P. infestans* airborne inoculum, a sensitive quantitative Polymerase Chain Reaction (qPCR) assay that can detect down to a single sporangium of *P. infestans* was developed. When *P. infestans* molecular assays [13, 29, 11] are evaluated for their specificity by including *P. phaseoli* and *P. mirabilis*, strong cross-reactions are often observed. The primers designed for this assay were sensitive to *P. mirabilis, P. phaseoli, P. ipomoeae* and *P. andina* since there is a high similarity among these species, sharing about 99.9 % of their ribosomal DNA internal transcribed spacer regions [30]. However, *P. mirabilis, P. ipomoeae* and *P. andina* are found mostly in Mexico and the Andes [31, 32] and there are no reports showing that they are adapted to the long distance aerial dispersion. Moreover, *P. mirabilis* and *P. ipomoeae* do not infect potatoes or tomatoes [31, 33]. The purpose of the qPCR tool is to detect and quantify airborne sporangia concentrations from potato fields or near to potato fields. In those circumstances, it can be expected that no other *Phytophthora* species or members of closely related taxa such as *Pythium* would interfere and bias the results obtained with the qPCR assay. The analytical sensitivity evaluation showed that DNA from *P. infestans* spores was efficiently extracted from silicone-coated rods. In all experiments, using the qPCR assay, it was possible to detect the expected number of sporangia with a detection sensitivity of one sporangium. Thus the method of extraction is optimized and the qPCR detection is

sensitive enough to detect very low numbers of sporangia. The fact that no signal was observed when rods were free from *P. infestans* sporangia is also an indication of the reliability of the assay. However, because the qPCR assay was validated only under laboratory conditions, field validation is still needed. Studies will be conducted over the next years to validate the qPCR assay with potato field samples.

This study addressed two main objectives derived from a previous study by Fall *et al.* [8], who, highlighted the need to determine infection efficiency and to develop a tool for quantifying *P. infestans* airborne sporangia in order to build a functional spore-sampling network to efficiently manage potato late blight. Overall, in term of number of lesions, clonal lineages US-23 showed higher aggressiveness than did the other clonal lineages tested (US-8, US-11 and US-24). An airborne sporangia concentration (ASC) of 10 sporangia m^{-3} for US-23 was sufficient to cause one lesion per leaf, whereas it took 15 to 25 sporangia m^{-3} for the other clonal lineages. According to these results, fungicides may be applied before the ASC reaches 1 sporangium m^{-3} in order to control potato late blight development. In addition to the traditional measurements, these results may be used to modulate the disease risk estimated by the decision support system. This study therefore provides an additional tool for late blight management and lays the groundwork for further studies to determine a field action threshold based on *P. infestans* ASC. To validate these findings under field conditions a qPCR assay was developed for quantifying *P. infestans* sporangia. Additional studies are currently underway to use airborne inoculum to determine which clonal lineage is present. Knowledge about both the amount of inoculum and the clonal lineage present can be used to adjust the fungicide scheme to the infection efficiency based on the aggressiveness of each clonal lineage and result in fewer fungicide applications without compromising the efficiency of late blight control.

4.6. Acknowledgements

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4.7. References

1. Fry WE, McGrath MT, Seaman A, Zitter TA, McLeod A, Danies G, Small IM, Myers K, Everts K, Gevens AJ, Gugino BK, Johnson SB, Judelson H, Ristaino J, Roberts P, Secor G, Seebold Jr KJ, Snover-Clift K, Wyenandt A, Grünwald NJ, Smart CD (2013) The 2009 late blight pandemic in Eastern USA – Causes and results. Plant Dis 97: 296–306.

2. Blair JE, Coffey MD, Martin FN (2012) Species tree estimation for the late blight pathogen, Phytophthora infestans, and close relatives. PLoS ONE 7: e37003. doi: 10.1371/journal.pone.0037003.

3. Haverkort AJ, Struik PC, Visser RGF, Jacobsen E (2009) Applied biotechnology to combat late blight in potato caused by Phytophthora infestans. Potato Res 52: 249–264.

4. Stein JM, Kirk WW (2001) Containment of existing potato late blight (Phytophthora infestans) foliar epidemics with fungicides. Crop Prot 21: 575–582.

5. Olanya OM, Honeycutt CW, Tschöepe B, Kleinhenz B, Lambert DH, Johnson SB (2012) Effectiveness of SIMBLIGHT1 and SIMPHYT1 models for predicting Phytophthora infestans in north-eastern United States. Arch Phytopathol Plant Prot 45: 1558–1569.

6. Skelsey P (2008) Multi-scale modeling of potato late blight epidemics. Ph.D. Thesis, Wageningen University. Available: http://edepot.wur.nl/122020. Accessed 23 June 2014.

7. Aylor DE, Schmale III DG, Shields EJ, Newcomb M, Nappo CJ (2011) Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agric For Meteorol 151: 251–260.

8. Fall, ML, Van der Heyden H, Brodeur L, Leclerc Y, Moreau G, Carisse O (2014) Spatiotemporal variation in airborne sporangia of Phytophthora infestans: Characterization and initiatives toward improving potato late blight risk estimation. Plant Pathol. doi: 10.1111/ppa.12235.

9. Haas BJ, Kamoun S, Zody MC, Jiang RHY, Handsaker RE, Cano LM, et al. (2009) Genome sequence and analysis of the Irish potato famine pathogen Phytophthora infestans. Nature 461: 393–398.

10. Cooke DEL, Lees AK (2004) Markers, old and new, for examining Phytophthora infestans diversity. Plant Pathol 53: 692–704.

11. Judelson HS, Tooley PW (2000) Enhanced polymerase chain reaction methods for detecting and quantifying Phytophthora infestans in plants. Phytopathology 90: 1112–1119.

12. Abbott CL, Gilmore SR, Lewis CT, Chapados JT, Peters RD, Platt HW, Coffey MD, Lévesque CA (2010) Development of a SNP genetic marker system based on variation in microsatellite flanking regions of Phytophthora infestans. Can J Plant Pathol 32: 440–457.

13. Tooley PW, Bunyard BA, Carras MM, Hatziloukas E (1997) Development of PCR primers from internal transcribed spacer region 2 for detection of Phytophthora species infecting potatoes. Appl Environ Microbiol 63: 1467–1475.

14. Llorente B, Bravo-Almonacid F, Cvitanich C, Orlowska E, Torres HN, Flawiá MM, Alonso GD (2010) A quantitative real-time PCR method for in planta monitoring of Phytophthora infestans growth. Lett Appl Microbiol 51: 603–610.

15. Bilodeau GJ, Lévesque CA, de Cock AWAM, Duchaine C, Brière S, Uribe P, Martin FN, Hamelin RC (2007) Molecular detection of Phytophthora ramorum by real-time polymerase chain reaction using TaqMan, SYBR Green, and molecular beacons. Phytopathology 97: 632–642.

16. Gisi U, Cohen Y (1996) Resistance to phenylamide fungicides: A case study with Phytophthora infestans involving mating type and race structure. Annu Rev Phytopathol 34: 549–572.

17. Danies G, Small IM, Myers K, Childers R, Fry WE (2013) Phenotypic characterization of recent clonal lineages of *Phytophthora infestans* in the United States. Plant Dis 97: 873–881.

18. Skelsey P, Rossing WAH, Kessel GJT, van der Werf W (2009) Scenario approach for assessing the utility of dispersal information in decision support for aerially spread plant pathogens, applied to *Phytophthora infestans*. Phytopathology 99: 887–895.

19. Kalischuk ML, Al-Mughrabi KI, Peters RD, Howard RJ, Platt HW, Kawchuk LM (2012) Genetic composition of Phytophthora infestans in Canada reveals migration and increased diversity. Plant Dis 96: 1729–1735.

20. Cohen Y (1994) Local and systemic control of Phytophthora infestans in tomato plants by DL-3-amino-n-butanoic acids. Phytopathology 84: 55–59.

21. Andreu AB, Caldiz DO (2006) Early management of late blight (Phytophthora infestans) by using systemic fungicides applied to seed-potato tubers. Crop Prot 25: 281–286.

22. Madden LV, Hughes G, van den Bosch F (2007) The study of plant disease epidemics. St. Paul, MN, USA: American Phytopathological Society. 421 p.

23. Logan M (2010) Biostatistical design and analysis using R: A practical guide. Chichester, UK: Wiley-Blackwell. 546 p.

24. Robideau GP, de Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, et al. (2011) DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. Molec Ecol Resour 11: 1002–1011.

25. Carisse O, Tremblay DM, Lévesque CA, Gindro K, Ward P, Houde A (2009) Development of a TaqMan real-time PCR assay for quantification of airborne conidia of Botrytis squamosa and management of Botrytis leaf blight of onion. Phytopathology 99: 1273–1280.

26. Seidl Johnson AC, Jordan SA, Gevens AJ (2014). Novel resistance in heirloom tomatoes and effectiveness of resistance in hybrids to Phytophthora infestans US-22, US-23, and US-24 clonal lineages. Plant Dis 98: 761–765.

27. Shakya SK, Goss EM, Dufault NS, van Bruggen AHC (2014). Potential effects of diurnal temperature oscillations on potato late blight with special reference to climate change. Phytopathology. doi: 10.1094/PHYTO-05-14-0132-R.

28. Refardt D, Ebert D (2006) Quantitative PCR to detect, discriminate and quantify intracellular parasites in their host: An example from three microsporidians in Daphnia. Parasitology 133: 11–18.

29. Lees AK, Sullivan L, Lynott JS, Cullen DW (2012) Development of a quantitative real–time PCR assay for Phytophthora infestans and its applicability to leaf, tuber and soil samples. Plant Pathol 61: 867–876.

30. Raffaele S, Farrer RA, Cano LM, Studholme DJ, MacLean D, Thines M, Jiang RHY, Zody MC, Kunjeti SG, Donofrio NM, Meyers BC, Nusbaum C, Kamoun S (2010) Genome evolution following host jumps in the Irish potato famine pathogen lineage. Science 330: 1540–1543.

31. Goss EM, Cardenas ME, Myers K, Forbes GA, Fry WE, Restrepo S, Grünwald NJ (2011) The plant pathogen Phytophthora andina emerged via hybridization of an unknown Phytophthora species and the Irish potato famine pathogen, P. infestans. PLoS ONE 6: e24543. doi: 10.1371/journal.pone.0024543.

32. Adler NE, Erselius LJ, Chacón MG, Flier WG, Ordoñez ME, Kroon LPNM, Forbes GA (2004) Genetic diversity of Phytophthora infestans sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. Phytopathology 94: 154–162.

33. Grünwald NJ, Flier WG (2005) The biology of Phytophthora infestans at its center of origin. Annu Rev Phytopathol 43: 171–190.

4.8. SUPPORTING INFORMATION LEGENDS

Additional supporting information may be found in the online version of this article.

Table S1. Exogenous internal positive control (EIPC) sequence used for detection inhibition of the qPCR reaction.

EIPC fragment sequence
TGGCACTAGAGCTGCCTGTTTCTATAGGGGGATGGGTTTTGAAAAACACTTGAT
GGGTGGGAGGACTGCATTCGAGCTGAGCGTCTCCAGAGTAAAGTACATCTCCAA
CGATAAATGTAACTGGTGATCCTCTGAGTCCACCCCCTAAAGGCTAGCTAGGAC
CGATCAATAGGGATAGGCAAGGTCGTCACCTCGGAACGCATAGGAACTGCTGAC
GAGAATTAAGGTCACTTTCGTAACGAAGCACTTTTCTTGCCTGTTCGCCGCTGAG
TAGCCACGTTATTATCGTCGCACCCAGATCGGGTATGAAGGGTGTAGGACAAGT
GCGGGACACAATCAAGTTAGTTGTTCGCGCCACGAGAAATCCGTGAAACAATAC
TAAGACACACAGGACTCTGAAAGCCGCTTGTATAACTCATGTTCCGGAGGCCCG
TTTCTCACGTTCGGGTTCTAGTCGCAGGGACAGAGGAAATCACGTTCGGTTGCG
GTTCTGTGCTTGG

CHAPITRE 5

SIMULATION DYNAMIQUE ET QUANTITATIVE DE LA CONCENTRATION AÉRIENNE DE CONIDIE DE *BREMIA LACTUCAEA*

Résumé

Le mildiou de la laitue (*Lactuca sativa L.*), causé par *Bremia lactucae* Regel, un oomycète, est une menace majeure dans la production mondiale de laitue. Le mildiou de la laitue est une maladie polycyclique où les spores aéroportées jouent un rôle primordial. Un modèle de simulation dynamique basé sur les conditions météorologiques a été développé pour simuler les caractéristiques aérobiologiques de *B. lactucae*. Le modèle a été construit en utilisant la plate-forme de STELLA et en suivant la méthodologie spécifique aux systèmes dynamiques. Le modèle a été développé en utilisant des équations publiées décrivant les sous-processus (par exemple, la sporulation) et les connaissances sur les interactions entre les agents pathogènes, leurs hôtes, et sur les conditions climatiques qui les affectent. Le modèle a été évalué avec quatre années de données en comparant les concentrations horaires et quotidiennes de spores simulées à partir du modèle avec les concentrations aériennes de spores observées. Le modèle simule presque parfaitement la tendance et la forme de la courbe qui caractérise la dynamique de la fluctuation des conidies de *B. lactucae*. Le modèle prédit les pics de concentrations aériennes de conidies. Dans plus de 95% du temps, la concentration aérienne quotidienne de conidies simulée était 0 lorsque la concentration aérienne de conidies observée était nulle. De même, la relation entre la concentration aérienne de conidies simulée et la concentration aérienne de conidies observée est linéaire. Dans plus de

95% des simulations, la proportion de la variation linéaire dans les valeurs observées qui est expliquée par la variation des valeurs simulées est supérieure à 0,7. La plupart des erreurs du modèle provenaient de sa déviation par rapport à la ligne 1:1, et la proportion des erreurs dues à un biais était faible. Ce modèle est l'un des rares modèles dynamiques développés pour mimer la dynamique de l'inoculum aérien et représente un premier pas vers l'amélioration des prévisions des épisodes de mildiou de la laitue ainsi que sa gestion.

La portée de l'article et les contributions respectives des auteurs

Le manuscrit publié dans Plant Pathology démontre l'importance de prendre en considération la présence et la quantité de spores aéroportées dans les systèmes de support décisionnel (SSD). Ce présent manuscrit démontre pour une première fois dans la littérature scientifique publiée qu'il est possible de simuler les concentrations aériennes de spores. L'implantation d'un réseau de capteur de spores peut être couteuse en termes de temps et d'argent dépendamment du nombre de capteurs requis. Par ailleurs, à défaut de remplacer le réseau de capteur de spores, l'utilisation de ce modèle de simulation pourrait permettre de réduire le nombre de capteur requis.

La contribution des auteurs dans cet article est comme suit : J'ai conceptualisé le modèle et j'ai effectué toutes les analyses statistiques, la rédaction du manuscrit ainsi que les figures et tableaux pour la publication. M^{r} van der Heyden et D^{r} Carisse ont agi comme conseillers, et ont révisé l'article pour sa publication. D^{r} Carisse a fourni les données de validation du modèle. Cette recherche a été sous la supervision de la D^{r} Carisse.

Les travaux présentés dans ce chapitre seront soumis dans **PlosOne**: Fall ML, van der Heyden H, and Carisse O (2015). A quantitative dynamic simulation of *Bremia lactucae* airborne conidia concentration above lettuce canopy. PlosOne (En révision).

A QUANTITATIVE DYNAMIC SIMULATION OF *BREMIA LACTUCAE* AIRBORNE CONIDIA CONCENTRATION ABOVE A LETTUCE CANOPY

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5.1. Abstract

Lettuce downy mildew, caused by the oomycete *Bremia lactucae* Regel, is a major threat to lettuce production worldwide. Lettuce downy mildew is a polycyclic disease driven by airborne spores. A weather-based dynamic simulation model for *B. lactucae* airborne spores was developed to simulate the aerobiological characteristics of the pathogen. The model was built using the STELLA platform by following the system dynamics methodology. The model was developed using published equations describing disease subprocesses (e.g., sporulation) and assembled knowledge of the interactions among pathogen, host, and weather. The model was evaluated with four years of independent data by comparing model simulations with observations of hourly and daily airborne spore concentrations. The results show an accurate simulation of the trend and shape of *B. lactucae* temporal dynamics of airborne spore concentration. The model simulated hourly and daily peaks in airborne spore concentrations. More than 95% of the simulation runs, the daily-simulated airborne

conidia concentration was 0 when airborne conidia were not observed. Also, the relationship between the simulated and the observed airborne spores was linear. In more than 94% of the simulation runs, the proportion of the linear variation in the hourly-observed values explained by the variation in the hourly-simulated values was greater than 0.7 in all years except one. Most of the errors came from the deviation from the 1:1 line, and the proportion of errors due to the model bias was low. This model is the only dynamic model developed to mimic the dynamics of airborne inoculum and represents an initial step towards improved lettuce downy mildew, understanding, forecasting and management.

Keywords: Decision-support system, Aerobiology, Downy mildew disease, Asexual spores, oomycetes

5.2. Introduction

Lettuce (*Lactuca sativa* L.) downy mildew, caused by the oomycete *Bremia lactucae* Regel, is a major threat to lettuce production around the world [1-2]. The life cycle of *B. lactucae,* an obligate biotrophic parasite, involves primary (sexual) and secondary (asexual) infection cycles. The sexual cycle results in soilborne oospores that are the likely source of primary inoculum [3]. Oospores can potentially overwinter and become a source of primary inoculum during the spring. However, there are no published data showing that *B. lactucae* oospores survive the harsh Canadian winter. The asexual cycle generates conidia that are adapted to aerial dispersal [4-5]. The conidia are produced when humidity is high and wind speed is low, and they are released in response to concomitant decreasing humidity and increasing temperature [6]. Viable conidia that land on leaves of susceptible lettuce plants germinate and colonize the leaves, resulting in symptoms that are visible 7 to 14 d after the initiation of infection [7]. First infection is then followed by successive asexual cycles occurring

throughout the lettuce production season. Environmental factors such as temperature, relative humidity (RH), wind speed, solar radiation, and leaf wetness duration have been identified as factors that determine the extent of conidia production, dispersal, and survival as well as the infection processes [4, 6, 8, 9, 10]. Hence, conidia survival is greater at 23°C (12h) than at 31°C (2 to 5 h), regardless of RH (33% and 76%), and conidia survival increases substantially at an RH of 90% or greater [5, 9]. Wind speed plays a major role in the conidia dispersal process, and solar radiation determines the survival of the airborne conidia [5, 7]. However, the most important factor for successful infection is the duration of morning and evening leaf wetness [11-15].

The control strategy for lettuce downy mildew is based mostly on chemical protection with fungicide applied at fixed or weather-based intervals. A difficulty that most growers face is identifying the best timing for fungicide applications to get the optimum level of control against downy mildew. Therefore, in the province of Quebec, Canada, fungicides are applied routinely to control downy mildew even though some of these applications may be unnecessary. In the pathosystem formed by lettuce and *B. lactucae*, two decision-support systems (DSSs) were developed to guide decision-making concerning the timing of fungicide applications. The first DSS, which was developed in California, USA, and was later modified, is based on leaf wetness ending late in the morning (10:00 hours) to predict when infection by *B. lactucae* occurs [13]. The second DSS, BREMCAST, was developed in Quebec, Canada [12], use a leaf wetness duration of 3 to 5 h after dawn (continuing until 10:00 hours) as an action threshold for fungicide application. Therefore, in both of these DSSs, leaf wetness duration is used as an indicator for occurrence of an infection event [12-13, 16]. These systems assume that sporulation is nocturnal, that conidia are released at dawn, and that infections occur in the morning [12-13]. However, the major limitation of these DSSs is their inability to assess the presence and amount of conidia above lettuce fields, and consequently these systems can overestimate or underestimated

the risk of downy mildew infection [17]. In fact, to evaluate the availability of *B. lactucae* airborne inoculum, these DSSs rely on signs of downy mildew in the field during routine scouting [12].

The potential risk of lettuce downy mildew development and consequent yield losses are related to the quantity of *B. lactucae* airborne conidia [15]. Fall et al. [15] observed a quantitative relationship between the airborne conidia concentration (ACC) of *B. lactucae* and the number of lesions per leaf. Hence, an ACC of 14 conidia/m^3 can cause one lesion per leaf [15]. Therefore, incorporating *B. lactucae* airborne conidia concentration into DSSs may help to develop more effective strategies for controlling lettuce downy mildew with better timing of fungicide applications [15].

The amount of airborne conidia above lettuce fields is dictated by several factors, including the sporulation intensity of sporulating lesions (source), the proportion of conidia that is released, the proportion of conidia that escapes the canopy layer and becomes airborne, and the proportion of surviving conidia that are deposited on susceptible lettuce leaves. Because each of these factors has been mathematically described for *B. lactucae* or closely related species [12-13, 18-19], these mathematical relationships could be used to simulate the quantity of airborne conidia.

Despite the amount of scientific information available on the aerobiology of plant pathogens, few published studies have actually focused on the simulation of airborne inoculum, even though it has been documented that inoculum is a key factor for the development of epidemics of several plant diseases [20-22].

In this study, we developed a dynamic simulation model of *B. lactucae* airborne conidia in an effort to improve the decision-making process for lettuce downy mildew management. The specific objectives of this study were (1) to develop a structurally dynamic model to simulate *B. lactucae* airborne conidia and (2) to assess the accuracy and sensitivity of the model against measured airborne conidia data.

5.3. Materials and methods

5.3.1. Asexual disease cycle processes

Because of the absence of information and evidence about the overwintering of oospores under Canadian weather conditions, the asexual life cycle of *B. lactucae* was used to build the model framework. This cycle can be divided in four major stages: the sporulation and release stage, the escape and dispersal stage, the survival and deposition stage, and the infection and germination stage. First, conidia that land on the leaves of susceptible lettuce plants germinate and colonize the leaf cells. The resulting infected leaves produce conidia under conditions of high humidity and low wind speed. These conidia are released in response to decreasing humidity and increasing temperature (sporulation and release stage). Second, increases in wind speed promote the escape of conidia from the canopy layer and the dissemination of these conidia (escape and dispersal stage). Third, weather variables, including solar radiation, temperature, and air RH, determine conidia survival, and as the wind speed decreases, the conidia are deposited gradually on lettuce leaves (survival and deposition stage). Finally, new infections by conidia require free water on leaf surfaces for germination (infection and germination stage) [8, 15]. Figure 5.1 shows the different stages of the asexual life cycle of *B. lactucae*.

5.3.2. Modelling approach

System dynamics is a methodology for studying and managing complex systems that change over time [23]. A model was built using the system dynamics methodology to simulate the asexual life cycle of *B. lactucae*. The model was developed according to the principles of “flow charts” in a dynamic simulation system

STELLA is a flexible computer modeling package with an easy, intuitive interface that allows users to construct dynamic models that can simulate biological systems. The main components of STELLA are stocks, flows, connectors, and converters. Stocks are accumulations within the system and can be different types, including reservoirs and conveyors. Flows are the movement of the stocks throughout the system and allow resources to be transported around the model. Connectors provide information links within the system, and converters contain the algebraic relationships within a model [23]. The change in any stock at a given time is expressed as follows:

$$\text{Stocks}(t) = \text{Stocks}(t - dt) + (n*\text{Inflow} - n*\text{outflow}) * dt \qquad (1)$$

where *t* is time, *dt* is differential of t, and *n* is number of inflows or outflows.

The simulation time unit is hour, and the variable inputs are estimated on an hourly basis. To reduce potential integration error, a built-in simulation algorithm of the Euler integration method and a simulation time step of 0.25 are used [23]. As a result, for each hour of simulation, the STELLA program runs four times for the integration process. The time horizon of the model is 24 h (1 d), and the model simulates the airborne conidia of *B. lactucae* from July to September of each year, which is the critical period for lettuce downy mildew in Quebec, Canada.

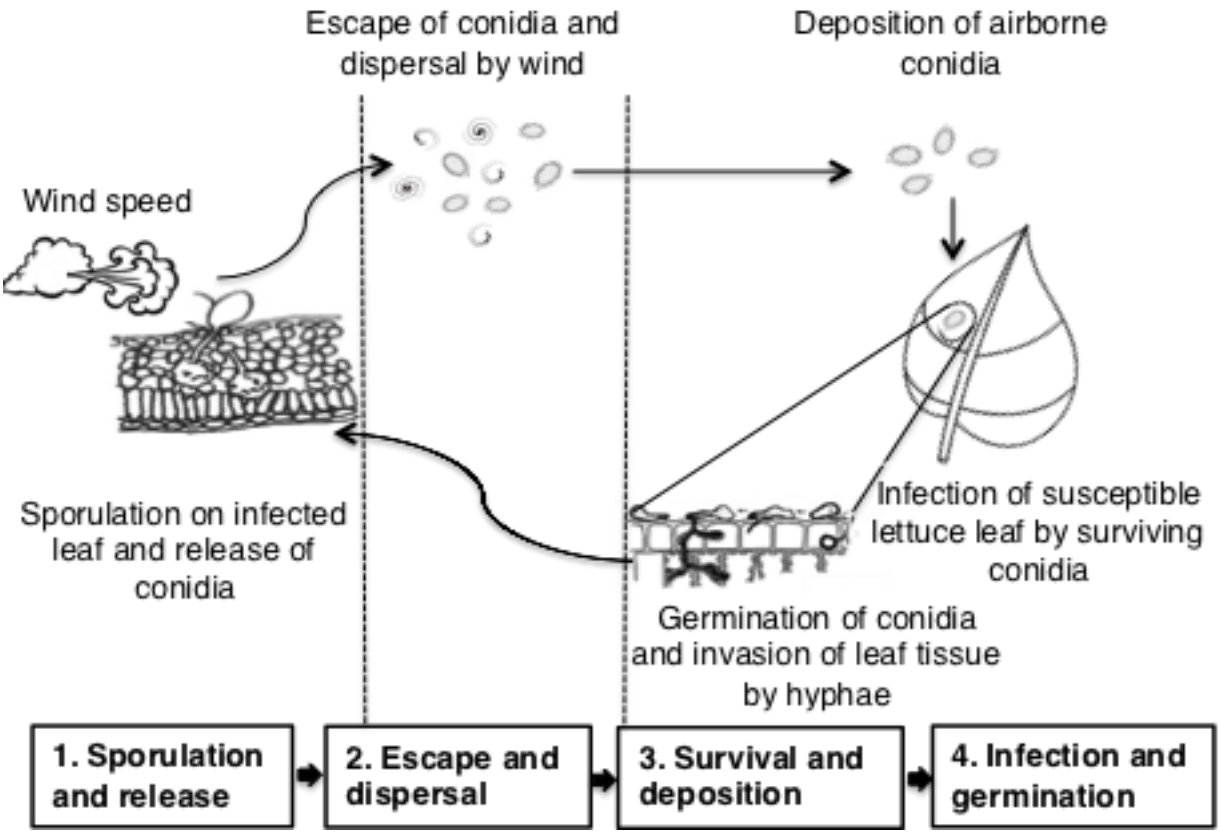

Figure 5. 1. Asexual stages of the life cycle of *Bremia lactucae*

5.3.3. Model description

The model can be divided into six state variables (stocks) that are linked by flow charts and connectors (Fig. 5.2, Table 5.1). The first state variable is the potential for the sporulation of one lesion (PSL). It is assumed that at least one lesion per m^2 of crop is sporulating when the temperature is between 5 and 25°C and the leaf wetness duration is greater than or equal to 2 h [15, 24]. It is also assumed that a sporulating lesion can produce a maximum of 20,000 conidia (unpublished data) and that the mean lesion size is 0.004 m^2 [25].

The second state variable is the relative number of released conidia (RNRC). The change in RNRC is dictated by the conidia inflow (released conidia), the conidia outflow (escaped conidia), and the conidia death rate, as follows:

RNRC(t) = RNRC(t − dt) + (Released− Escaped− Conidia deaths) * dt (2)

where *t* is time, *dt* is differential of t. The algebraic equations describing conidia inflow and conidia outflow for Eq. 2 are defined in Table 5.2.

The third state variable is the relative number of escaped conidia (RNEC). The change in RNEC is dictated by the conidia inflow (escaped conidia), the conidia outflow (surviving conidia), and the conidia death rate, as follows:

RNEC(t) = RNEC(t − dt) + (Escaped − Surviving − Conidia deaths) * dt (3)

where *t* is time, *dt* is differential of t. The algebraic equations describing conidia inflow and outflow for Eq. 3 are defined in Table 5.2.

The fourth state variable is the relative number of surviving conidia (RNSC). The change in RNSC is a function of conidia survival and conidia deposition, as follows:

RNSC(t) = RNSC(t − dt) + (Surviving − Death − Deposited) * dt (4)

where *t* is time, *dt* is differential of t.

Table 5. 1. Description of variables and parameters used in the model

Abbreviation	Description	Unit
PSL	Potential for the sporulation of one lesion per m^2	Number (=1)
RNRC	Relative number of released conidia per m^2	Number (0 to ∞)
RNEC	Relative number of escaped conidia per m^2	Number (0 to ∞)
RNSC	Relative number of survived conidia per m^3	Number (0 to ∞)
RNDC	Relative number of deposited conidia per m^2	Number (0 to ∞)
RNGC	Relative number of germinated conidia per m^2	Number (0 to ∞)
Sp	Sporulation	Number (0 to ∞)
Escp rate	Escape rate	Number (0 to ∞)
Surv rate	Survival rate	Number (0 to ∞)
Germ	Germination	Number (0 to ∞)
UEC	Unescaped conidia	Number (0 to ∞)
UDC	Undeposited conidia	Number (0 to ∞)
Asymp1	Asymptote for sporulation equation	Number (0 to ∞)
Asymp2	Asymptote for germination equation	Number (0 to ∞)
r1	Rate for sporulation equation	0.75
r2	Rate for germination equation	Number (0 to ∞)
Ws	Wind speed	m/s
SR	Solar radiation	MJ/m^2
RH	Relative humidity	%
LWD	Leaf wetness duration	H
Tp	Average temperature over leaf wetness duration	°C

The fifth state variable is the relative number of deposited conidia (RNDC), described as follows:

$$RNDC(t) = RNDC(t - dt) + (Deposited) * dt \qquad (5)$$

where *t* is time, *dt* is differential of t and the variables are defined in Table 5.2.

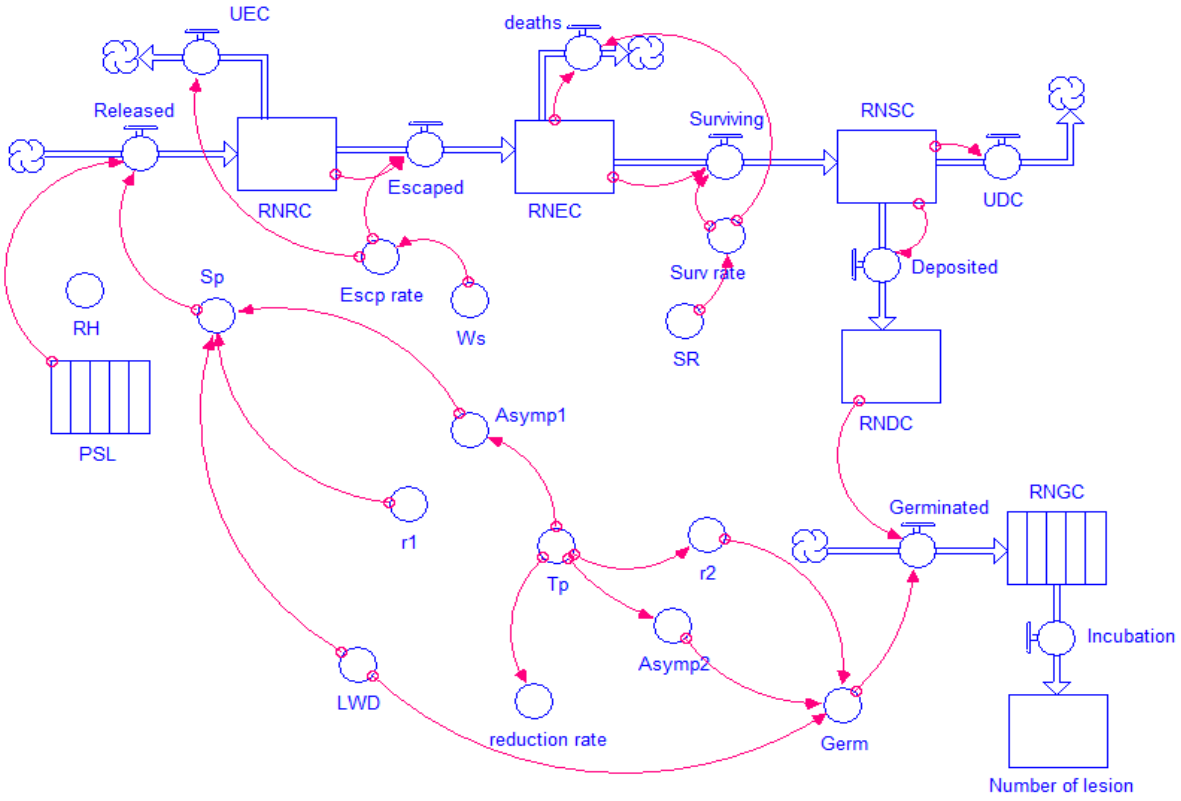

Figure 5.2. Diagram of the model predicting *Bremia lactucae* airborne conidia. Legend: ▭ state variable (simple stock); ▥ state variable (conveyor stock); ⧑ inflow or outflow from stock; ⇢ parameter information flow; ○ parameter (Table 5.1 for acronyms descriptions).

The sixth state variable is the relative number of germinated conidia (RNGC), described as follows:

$$RNGC(t) = RNGC(t - dt) + (Germinated - Incubation) * dt \quad (6)$$

where *t* is time, *dt* is differential of t and the variables are defined in Table 5.2.

The germination process and the duration of incubation dictate the changes in the RNGC. The duration of incubation is defined as the time from spore deposition to

lesion production and is described in Table 5.2. Running the model requires weather variables, including leaf wetness duration, RH, temperature, wind speed, and solar radiation (Table 5.1). Each of the six state variables can be chosen as model outputs.

5.3.4. Measurement of environmental variables

Leaf wetness duration was assessed every 15 minutes with electrical-impedance leaf-wetness sensors (Model 237; Campbell Scientific, Edmonton, AB, Canada) placed at the height of the lettuce leaves. In 1997 and 1998, air temperature (°C) was recorded with a data logger (Model 21X; Campbell Scientific) placed near the spore sampler, and RH (%) was monitored with a probe (Model HMP35C; Campbell Scientific). In 2003 and 2004, air temperature and RH were monitored using WatchDog data loggers (Spectrum Technologies, Aurora, IL, USA) placed near the spore sampler. Weather variables were monitored every 30 min, and hourly averages were used in the analyses. The temperature and RH probes were placed in a white shelter 1.5 m above the ground. For all years, wind speed (10 m above the ground) and solar radiation data were obtained from an Environment Canada weather station located approximately 200 m from the plots [7, 15].

5.3.5. Model evaluation

The model was evaluated using four years (1997, 1998, 2003, and 2004) of hourly data on *B. lactucae* ACC [7, 15]. The data were collected between 1 July and 20 September in each of 1998, 1997, 2003, and 2004. Each year, a plot of the lettuce cultivar Ithaca was established in an organic soil at the Agriculture and Agri-Food Canada experimental farm in Ste-Clotilde, QC, Canada (latitude 45°10′ N, longitude

73°40′ W). Lettuce plants produced in a greenhouse by Les Serres Lefort (Ste-Clotilde, QC, Canada) were transplanted 0.3 m apart in the rows, with 0.35 m between rows. A 7-d volumetric spore sampler (Burkard Manufacturing Co., Rickmansworth, Hertfordshire, UK) placed in the center of the plot was used to monitor ACC two weeks per month between July and September. The sampler was adjusted to sample air at 0.01 m^3/min. Impaction tapes were coated with a thin layer of silicone grease before they were placed in the sampler. Tapes were removed at 7-days intervals, cut into 0.048 m long segments corresponding to 24 h periods. Conidia were counted on whole transects perpendicular to the tape length. These transects were fixed at 0.002 m intervals to obtain hourly counts. Conidia counts were performed with a microscope at 250× magnification (0.00075 m wide transect) and converted to conidia per cubic meter of air for each hour of the day [7].

For each year, the hourly-simulated conidia concentrations were compared with the hourly-observed conidia concentrations for a period of 24 h. The cumulative conidia concentration within a 24-h period was considered the daily conidia concentration, and hence the daily-simulated conidia concentrations were compared with the daily-observed conidia concentrations.

In order for accuracy to be compared, regression of observed data (on the y-axis) versus simulated data (on the x-axis) was used instead of regression of simulated data (on the y-axis) versus observed data (on the x-axis) [28-29]. Also, when the coefficient of determination was greater than 0.7, Theil's decomposition of error was performed [30]. Theil's U statistic is decomposed into three coefficients of inequality (Eq. 7): mean differences between the observed and simulated airborne conidia, U_{bias}; deviations from the 1:1 line, U_{slope}; and the unexplained variance, U_{error}. The coefficients are calculated as follows:

$$U_{bias} = \frac{n(\bar{O} - \bar{S})^2}{\sum (O - S)^2}$$

$$U_{slope} = \frac{(b-1)^2 \sum (S - \bar{S})^2}{\sum (O - S)^2} \qquad (7)$$

$$U_{error} = \frac{\sum (\tilde{O} - O)^2}{\sum (O - S)^2}$$

where *n* is the number of simulations, *O* and *S* are the observed and predicted values of airborne conidia, respectively, $\bar{O}$ and $\bar{S}$ are the observed and predicted means, respectively, and *b* is the slope in the equation $\tilde{O}$ = a + bS, where $\tilde{O}$ is calculated observed values based on simulated values.The sum of the three coefficients is 1. A numerical sensitivity analysis was carried out to evaluate the sensitivity of the model to the weather variables (leaf wetness duration, sporulation rate [which is defined as a function of temperature; see Table 5.2], wind speed, solar radiation, and RH).

Table 5. 2. Algebraic equations describing the movement of the stocks throughout the model

Variable	Equation	Reference
Sp	$Asymp1*(1+25118.86*\exp(-r1*LWD))^{(-0.909)}$	Tchervenivanova, 1995
Asymp1	$0.966-0.000051*(Tp)^3-15.575/(Tp)^2$	Tchervenivanova, 1995
Germ	$Asymp2*\exp(-\exp(r2*(LWD-2)))$	Scherm and van Bruggen, 1993
Asymp2	$0.385+0.054*Tp-0.0024*Tp^2$	Scherm and van Bruggen, 1993
r2	$-1.154+0.327*Tp-0.011*Tp^2$	Scherm and van Bruggen, 1993
Escp rate	$0.073*Ws-0.0087$	Fall, unpublished data, 2013
Surv rate	IF SR<1 THEN 0.8 ELSE 0.6	Bhaskara Reddy et al., 1996
Released	$PSL*Sp$	
Escaped	$RNRC*Escp.rate$	
Surviving	$RNEC*Surv.rate$	
Deposited	$RNSC*1/30$	Kranz, 1974
Germinated	$RNDC*Germ$	
Incubation	240 h	Scherm and van Bruggen, 1993, 1994

5.4. Results

In the time window from July to September in 1997, 1998, 2003, and 2004, a total of 132 simulation runs were performed. Simulation runs (one run correspond to one day) were done 32, 36, 33, and 31 times in 1997, 1998, 2003, and 2004 respectively. There was a linear relationship between the hourly-observed number of conidia and the hourly-simulated number of conidia. In 1997, 1998, 2003, and 2004, the proportion of the linear variation in the hourly-observed values explained by the variation in the hourly-simulated values was greater than 0.7 (R^2) in 32.5%, 94.7%, 96.8%, and 100% of the total number of simulated days, respectively (Table 5.3, Figs. 5.3 and 5.4). The hourly-simulated and observed conidia concentrations had a similar trend and shape (Figs. 5.3 and 5.4).

There was a linear relationship between the daily-observed number of conidia and the daily-simulated number of conidia. In 1997, 1998, 2003, and 2004, the proportions of the linear variation in the daily-observed values explained by the variation in the daily-simulated values were 0.12, 0.88, 0.74, and 0.75, respectively (Table 5.4, Fig. 5.5). The trends and the conidia peaks in the daily-simulated and daily-observed conidia concentrations were similar in all years except 1997, when some simulated peaks in conidia were not observed (Fig. 5.6).

Table 5.3. Coefficients of determination of the regression model of hourly observed versus hourly simulated airborne conidia concentrations of *Bremia lactucae* in a lettuce field. *n*, number of simulations; R^2, coefficient of determination.

Year	n	Coefficients of determination				
		$R^2 > 0.9$	$0.8 < R^2 < 0.9$	$0.7 < R^2 < 0.8$	$0.6 < R^2 < 0.7$	$R^2 < 0.6$
1997	32	10.4%	15.3%	6.8%	36.8%	30.6%
1998	36	7.5%	10.6%	76.6%	5.3%	0.0%
2003	33	15.2%	16.3%	65.3%	3.0%	0.0%
2004	31	5.0%	30.9%	64.1%	0.0%	0.0%

Table 5.4. Evaluation of the regression model of daily observed versus daily simulated airborne conidia concentrations of *Bremia lactucae* in a lettuce field. *n*, number of simulations; R^2, coefficient of determination; U_{bias}, differences between the observed and predicted airborne conidia; U_{slope}, deviations from the 1:1 line; U_{error}, the unexplained variance.

Year	n	R^2	U_{bias}	U_{slope}	U_{error}
1997	32	0.12	-	-	-
1998	36	0.88	0.28	0.71	0.00
2003	33	0.74	0.14	0.85	0.00
2004	31	0.75	0.22	0.78	0.00

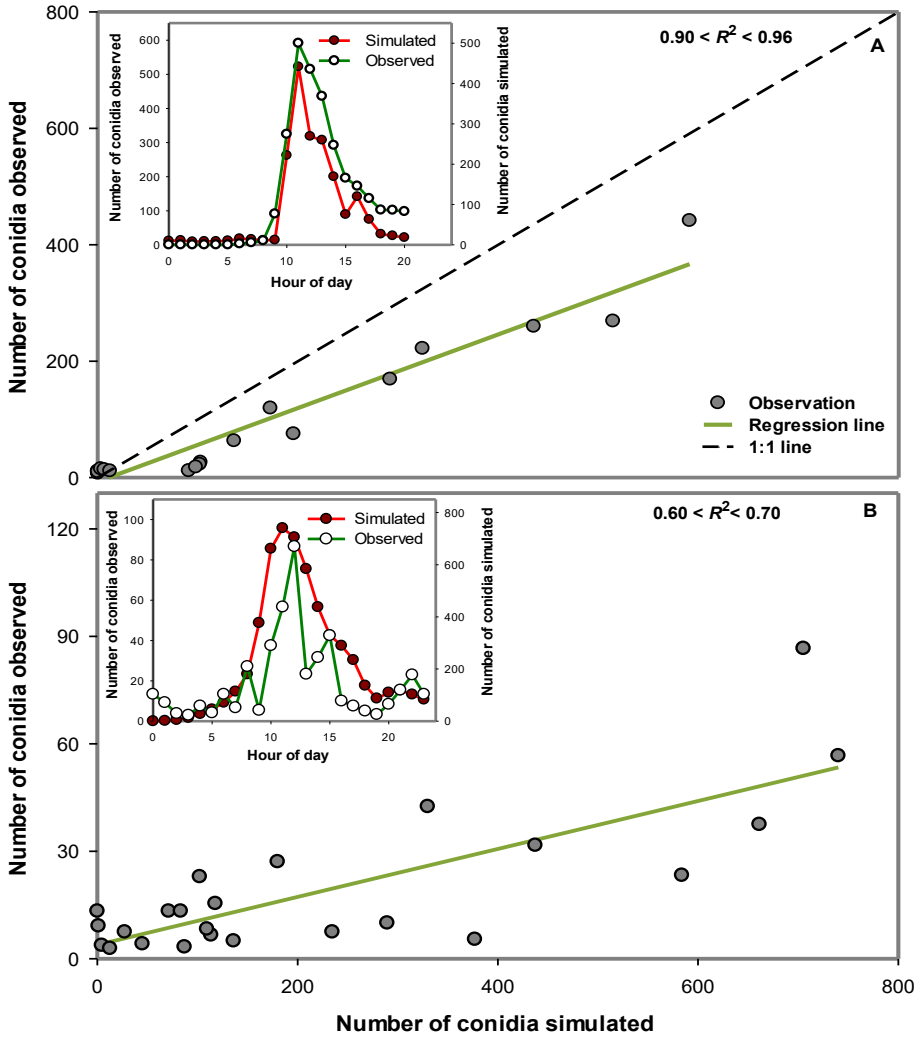

Figure 5.3. Examples of scatter plots of hourly simulated versus hourly observed airborne conidia concentrations of *Bremia lactucae* for the dynamic model developed to monitor airborne inoculum of the lettuce downy mildew pathogen. (A) Example of a case when $0.90 < R^2 < 0.96$, and (B) example of case when $0.60 < R^2 < 0.70$. The dashed line indicates 1:1 agreement between simulated and observed airborne conidia. The solid line indicates the fitted values from the regression of simulated versus observed airborne conidia. R^2, coefficient of determination. The inset graph in each panel represents the hourly observed airborne conidia as a function of hour of day airborne conidia.

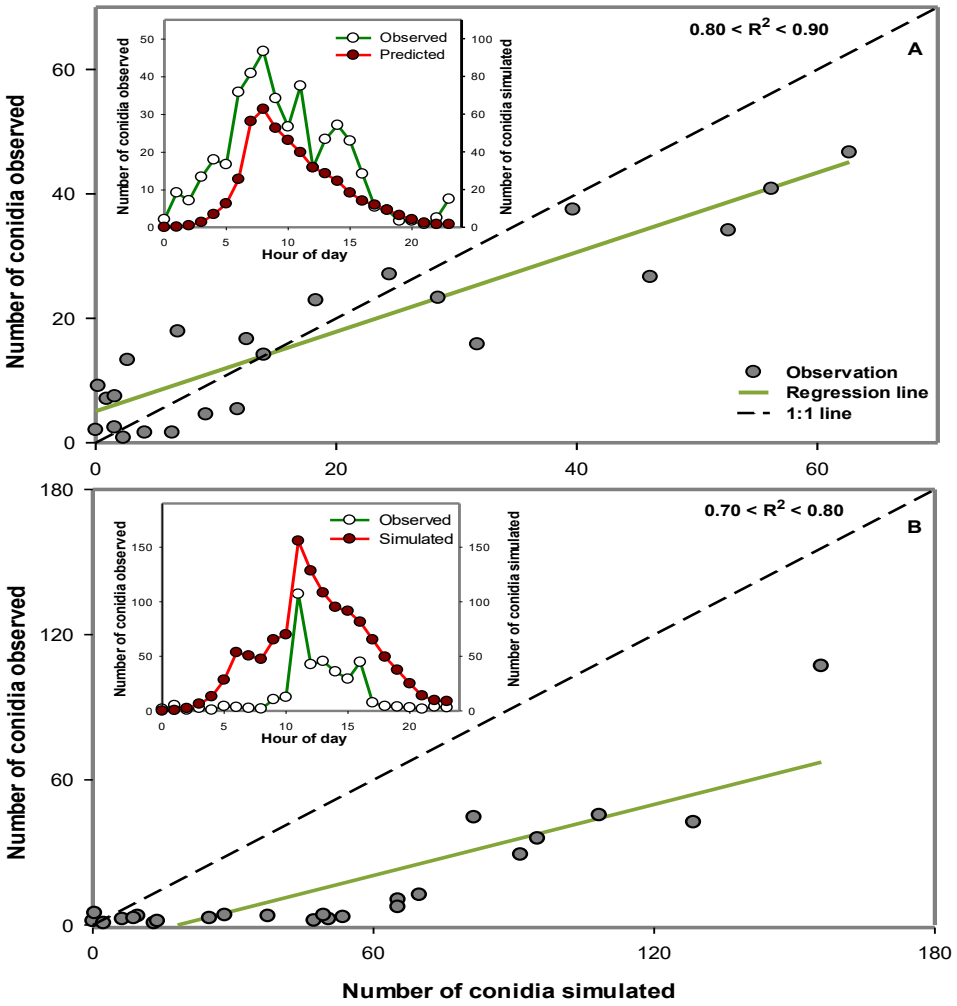

Figure 5.4. Examples of scatter plots of hourly simulated versus hourly observed airborne conidia concentrations of *Bremia lactucae* for the dynamic model developed to monitor airborne inoculum of the lettuce downy mildew pathogen. (A) Example of a case when $0.80 < R^2 < 0.90$, and (B) example of a case when $0.70 < R^2 < 0.80$. The dashed line indicates 1:1 agreement between simulated and observed airborne conidia. The solid line indicates the fitted values from the regression of simulated versus observed airborne conidia. R^2, coefficient of determination. The inset graph in each panel represents the hourly simulated and hourly observed airborne conidia as a function of hour of day.

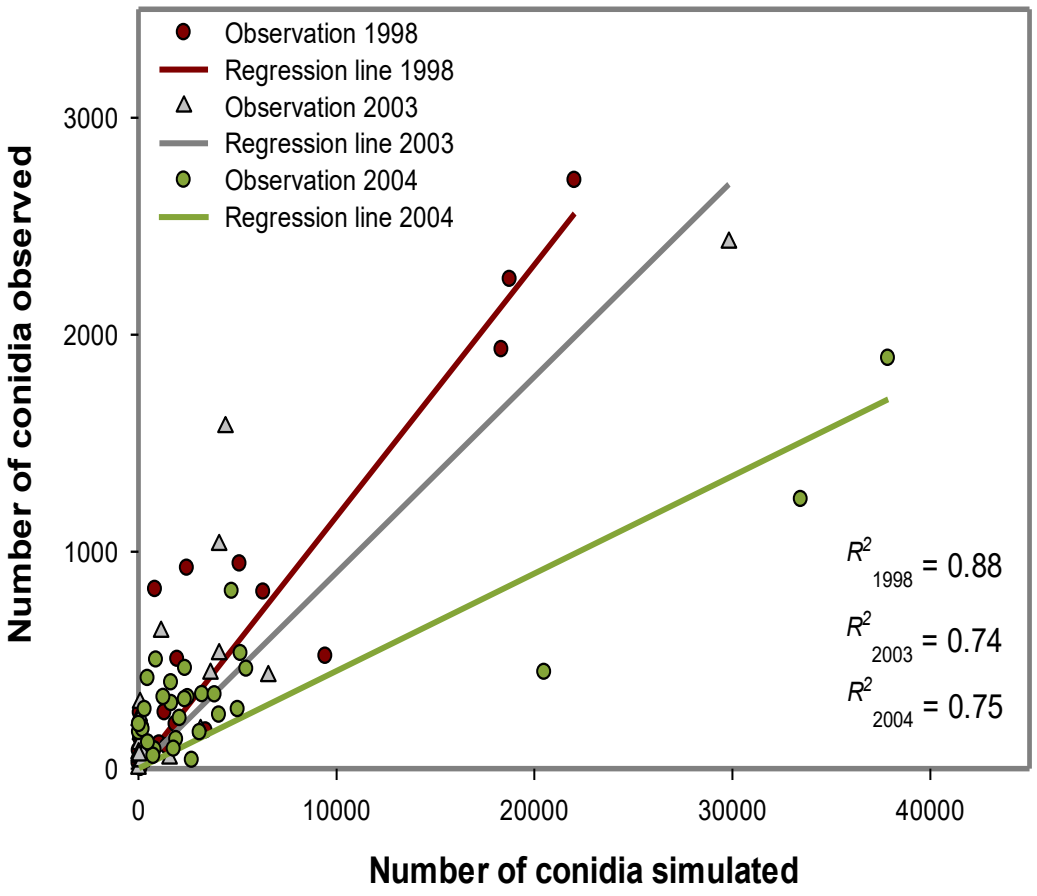

Figure 5.5. Regression analysis of daily simulated versus daily observed airborne conidia concentrations of *Bremia lactucae* in 1998, 2003, and 2004 for the dynamic model developed to monitor airborne inoculum of the lettuce downy mildew pathogen. The solid lines indicate the fitted values from the regressions of simulated versus observed airborne conidia. R^2, coefficient of determination.

Most of the errors in the daily-simulated conidia came from the deviations from the 1:1 line. In 1998, 71% of the error was due to the deviation from the 1:1 line, whereas the proportion of error associated with the bias was 3%. In 2003, 85% of the error

was due to the deviation from the 1:1 line, whereas the proportion of error associated with the bias was 14%. In 2004, 78% of the error was due to the deviation from the 1:1 line, whereas the proportion of error associated with the bias was 22% (Table 5.4). In 96%, 98%, 99%, and 99% of cases when no airborne conidia were measured, the daily-simulated airborne conidia concentrations were 0 in 1997, 1998, 2003, and 2004, respectively. Sensitivity analysis of the model showed that the model was highly sensitive to the rate of sporulation (r1 in the Sp equation in Table 5.2), which is the multiplication factor of spore production. Increasing the sporulation rate to one unit increased the simulated conidia number to 1.6 units (Fig. 5.7).

5.5. Discussion

Commercially acceptable control of lettuce downy mildew is achieved when only a few external leaves are infected. Regardless of the type of lettuce (head, leaf, or romaine), the commercialized parts must be free of disease. Because the development of lettuce downy mildew is strongly related to the environmental conditions [15], the decision support systems (DSS) that have been developed in the last 20 years rely on weather conditions to predict the best time for fungicide applications. However, epidemics are also driven by the presence and quantity of inoculum in the lettuce field [26]. Consequently, to improve the effectiveness of DSSs, it is crucial to find a way to simulate or measure airborne inoculum above the lettuce canopy [12, 31]. The results of recent studies [17, 31] suggested that inoculum could be monitored with a spore-sampling network. A high disease risk as estimated by a DSS in combination with a significant airborne spore concentration would trigger fungicide applications. However, there are advantages and limitations associated with both monitoring and simulating airborne inoculum. Spore sampling is generally representative of the real airborne inoculum concentration. However, because several samplers may be necessary to achieve an acceptable level of representativeness [17]

the cost and time required to obtain the information are increased, especially when the spore count is done by microscopy. In contrast, using a simulation model to estimate airborne inoculum is less costly and easier to implement once the model has been developed. However, the reliability of simulations is influenced considerably by the quality of the weather data used. The ideal situation is probably to combine the monitoring and simulation of airborne inoculum.

In this study, a weather-based simulation model of *B. lactucae* ACC was developed and validated with independent data (data not used to develop the model). For this model, the asexual life cycle of *B. lactucae* was divided into six state variables, and the changes from one state to the next were described using mathematical equations derived from the scientific literature or developed by assembling knowledge of the interactions between pathogen and host. The model was evaluated with four years of data by comparing model simulations with field observations of hourly and daily airborne conidia concentrations.

The model followed the trend and shape of hourly and daily-observed conidia concentrations almost perfectly. In over 94% of the simulation runs, the proportion of the linear variation in the hourly-observed values explained by the variation in the hourly-simulated values was greater than 0.7 in all years except in 1997. Over the four-year simulation period, the proportion of the linear variation in the daily-observed values explained by the variation in the daily-simulated values was greater than 0.70 in all years except in 1997. Also, most of the errors in the daily-simulated conidia came from the deviations from the 1:1 line, and the proportion of error associated with the model bias was low. Overall, the model was accurate in simulating the trend and the peaks in *B. lactucae* ACC, even though the model overestimated the daily number of conidia on some days. This situation is due probably to the model's sensitivity to the sporulation rate. Indeed, sensitivity analysis showed that the model was highly

sensitive to variation in the sporulation rate, which was defined as a function of temperature. Further field validations to calibrate the sporulation model developed by Tchervenivanova (1995) seem to be necessary.

These results can be useful for decision making to improve lettuce downy mildew management. Indeed, Fall et al. [15] found an exponential relationship between downy mildew intensity and the airborne conidia concentration (ACC). An ACC of 14 conidia/m^3 was required to cause one lesion per leaf in the field [15]. Over 95% of the time, when no airborne conidia were measured, the daily-simulated ACC was 0. Therefore, when the simulated number of airborne conidia is 0, fungicides should not be applied, whereas simulated peaks in airborne conidia should lead to fungicide applications. Indeed, the disease will develop only if the pathogen is present in the area [20, 22]. In the pathosystem formed by potato and *Phytophthora infestans*, an oomycete like *B. lactucae*, the first signs of disease were detected between 6 and 7 d after the peak in airborne spores [17, 32]. Thus, instead of waiting for signs of downy mildew in the field before running the BREMCAST DSS [12], the model developed in this study could be used. It may be risky to wait until downy mildew is noticed in the field before running a BREMCAST DSS. Thus, DSSs can be modified to incorporate simulated or measured airborne inoculum above the lettuce canopy. However, it will be a complex process to modify these DSSs without rebuilding the entire model because, these latter were not built using a dynamic system methodology. Also, one challenge involved in implementing a spore sampling network is to know whether the number of samplers is sufficient to obtain a representative airborne conidia concentration for the targeted area. In this context, it is probably better to combine monitoring and simulation of airborne inoculum instead of increasing the number of samplers, which comes at a cost.

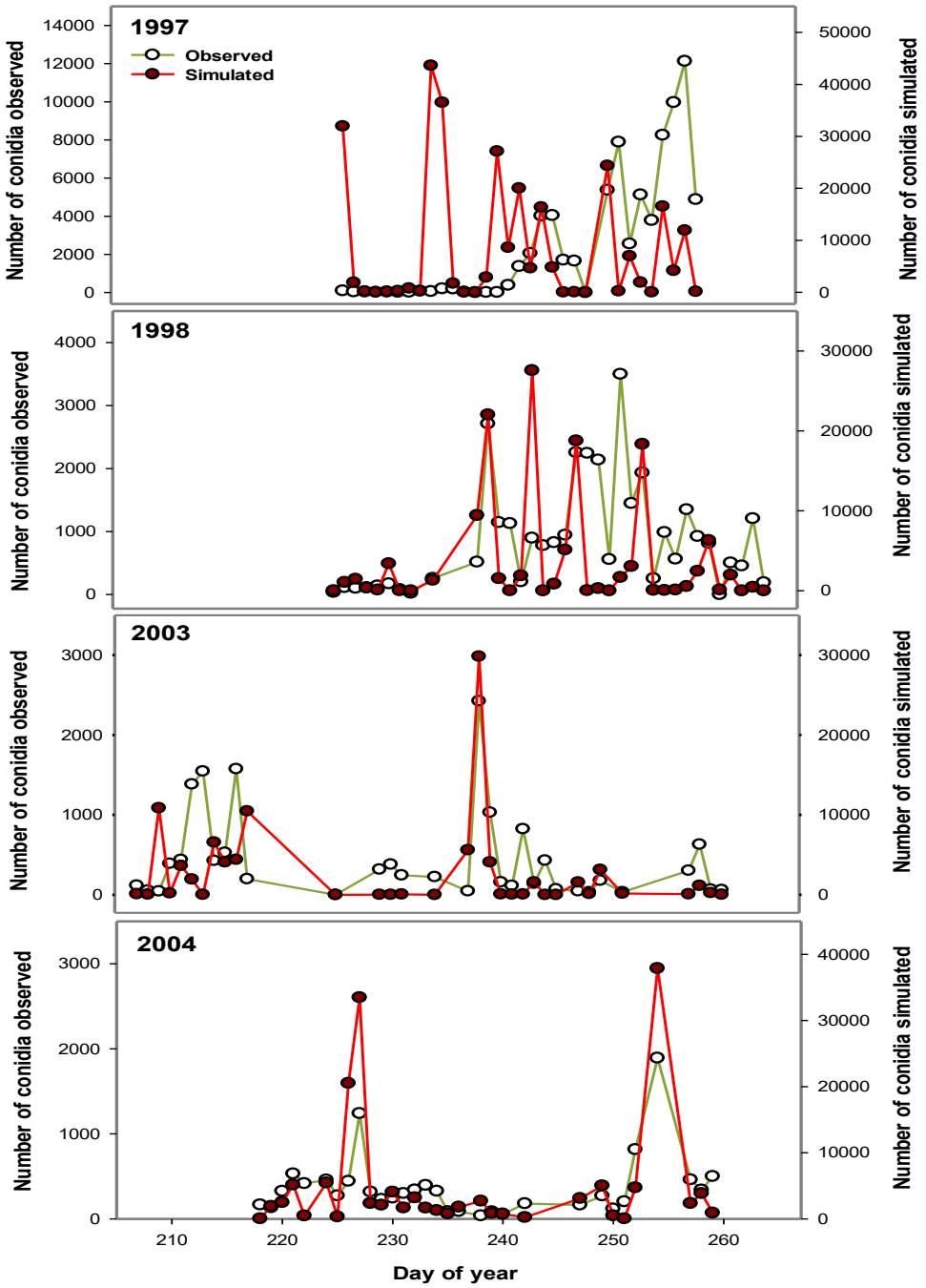

Figure 5.6. Daily observed and simulated daily airborne conidia of *Bremia lactucae* in 1997, 1998, 2003 and 2004, for the dynamic model developed for monitoring of lettuce downy mildew pathogen's airborne inoculum.

Nevertheless, the model missed some observed conidia on certain days. Also, some simulated peaks in conidia were not observed in 1997, and the simulations during that year were not accurate on most of the days. However, 1997 was the first year for the deployment of *B. lactucae* spore traps, and experience with conidia counts was lacking, and it is therefore possible that poor identification and incorrect counts occurred. The ACC is strongly related to the number of sporulating lesions across the field, and hence it is difficult to estimate the exact ACC. The uncertainties stemming from the highly variable potential number of lesions make it difficult to quantify the airborne inoculum at a large scale. At the moment, there are no methods for quantifying the number of sporulating lesions in a given set of weather conditions. Once such methods have been developed, it will be possible to circumscribe the model at a specific scale (small or large) and improve the accuracy of quantitative simulations of airborne conidia.

To the authors' knowledge, this is the first time in the published literature that a quantitative dynamic simulation model of airborne conidia was developed. The model offers advantages for accurately simulating the trend and the temporal progression of *B. lactucae* airborne conidia. Indeed, from a strictly epidemiological point of view, the focus is on the rate of change rather than the direct stage of the process. The modeling of epidemic of polycyclic disease such as lettuce downy mildew can be significantly improved by taking into account quantitative aspects of the asexual cycle of the pathogen. The existing DSSs in the literature did not take into consideration airborne conidia derived from the asexual life cycle of *B. lactucae*. The results obtained with this model compare favorably with field-observed data for airborne conidia. To the authors' knowledge, only the PLANT-Plus model developed by Dacom for the management of *Phytophthora infestans* integrates a submodel for airborne spores. Nonetheless, this submodel does not simulate the absolute number of spores; it just confirms their presence [33]. Moreover, the model developed in the present study may be used as a research tool for investigating the impact of

agricultural practices (e.g., irrigation systems) or weather conditions (e.g., temperature, wind speed, and RH) on airborne inoculum. The next step in the development of the model will be incorporating it into DSSs in order to more efficiently predict episodes of lettuce downy mildew.

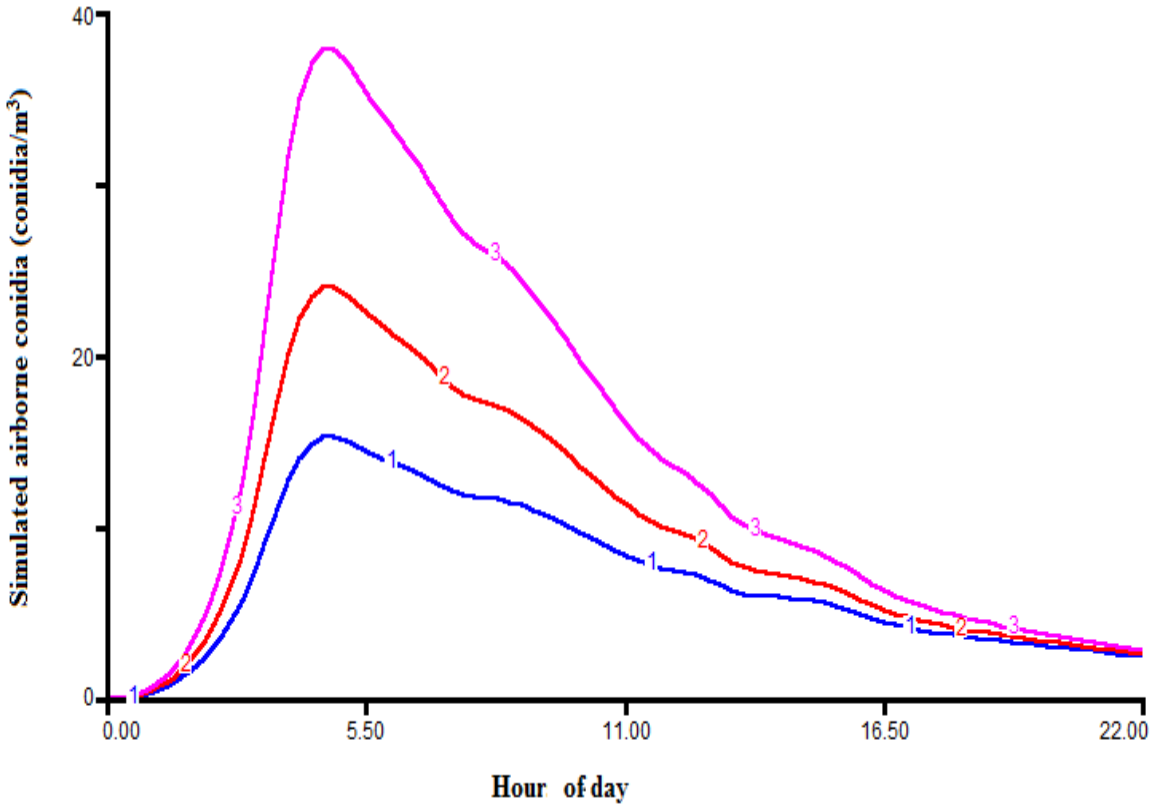

Figure 5.7. Sensitivity analysis of the model to the sporulation rate. The blue (1), red (2), and purple (3) lines represent rates of 0.5, 0.6, and 0.7, respectively.

5.6. Acknowledgments

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5.7. References

1. Lebeda A, Petrželová I (2004) Variation and distribution of virulence phenotypes of Bremia lactucae in natural populations of *Lactuca serriola*. Plant Pathology 53: 316–324.

2. Nordskog B, Gadoury DM, Seem RC, Hermansen (2007) A Impact of diurnal periodicity, temperature, and light on sporulation of *Bremia lactucae*. Phytopathology 97: 979–986.

3. Michelmore RW, Ilott TW, Hulbert SH, Farrara BF (1988) The downy mildews. In: Sidhu, G.S. (Ed.), Genetics of Plant Pathogenic Fungi, vol. 6, DS Ingram, PH Williams (Eds.), Advances in Plant Pathology series. Academic Press, London, UK pp. 53–79.

4. Fletcher JT (1976) Bremia lactucae, oospores, sporangial dissemination, and control. Ann. Appl. Biol. 84: 294–298.

5. Wu BM, Subbarao KV, van Bruggen AHC (2000) Factors affecting the survival of Bremia lactucae sporangia deposited on lettuce leaves. Phytopathology 90: 827–833.

6. Wu BM, van Bruggen AHC, Subbarao KV, Pennings GGH (2001) Spatial analysis of lettuce downy mildew using geostatistics and geographic information systems. Phytopathology 91: 134–142.

7. Carisse O, Philion V (2002) Meteorological factors affecting periodicity and concentration of airborne spores of *Bremia lactucae*. Can. J. Plant Pathol 24: 184–193.

8. Scherm H, van Bruggen AHC (1994) Weather variables associated with infection of lettuce by downy mildew (*Bremia lactucae*) in coastal California. Phytopathology 84: 860–865.

9. Su H, van Bruggen AHC, Subbarao KV, Scherm H (2004) Sporulation of Bremia lactucae affected by temperature, relative humidity, and wind in controlled conditions. Phytopathology 94: 396–401.

10. Wu BM, van Bruggen AHC, Subbarao KV, Scherm H, (2002) Incorporation of temperature and solar radiation thresholds to modify a lettuce downy mildew warning system. Phytopathology 92: 631–636.

11. Hovius MHY, McDonald MR, Carisse O, Philion V (2007) Field evaluation of weather-based spray programs for the control of downy mildew of lettuce (*Lactuca sativa*), caused by *Bremia lactucae*, in Quebec and Ontario. Can. J. Plant Pathol. 2007; 29: 9–17.

12. Kushalappa AC (2001) BREMCAST: Development of a system to forecast risk levels of downy mildew on lettuce (*Bremia lactucae*). Int. J. Pest Manage 47: 1–5.

13. Scherm H, Koike ST, Laemmlen FF, van Bruggen AHC (1995) Field evaluation of fungicide spray advisories against lettuce downy mildew (*Bremia lactucae*) based on measured or forecast morning leaf wetness. Plant Dis. 79: 511–516.

14. Su H, van Bruggen AHC, Subbarao KV (2000) Spore release of Bremia lactucae on lettuce is affected by timing of light initiation and decrease in relative humidity. Phytopathology 90: 67–71.

15. Fall ML, Van der Heyden H, Beaulieu C, Carisse O (2015) *Bremia lactucae* infection efficiency in lettuce is modulated by temperature and leaf wetness duration under Quebec field conditions. Plant Dis 99: 1010-1019. doi:10.1094/PDIS-05-14-0548.

16. Scherm H, van Bruggen AHC (1993) Sensitivity of simulated dew duration to meteorological variations in different climatic regions of California. Agric. For. Meteorol 66: 229–245.

17. Fall ML, van der Heyden H, Brodeur L, Leclerc Y, Moreau G, Carisse O (2015) Spatiotemporal variation in airborne sporangia of Phytophthora infestans: Characterization and initiative toward improving potato late blight risk estimation. Plant Pathol 64: 178–190. doi:10.1111/ppa.12235.

18. Tchervenivanova E (1995) Development of a model to predict sporulation of Bremia lactucae in lettuce. M.Sc. Thesis, McGill University, Montreal, QC, Canada. 77 pp.

19. Aylor DE, Fry WE, Mayton H, Andrade-Piedra JL (2001) Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. Phytopathology. 91: 1189–1196.

20. Skelsey P, Rossing WAH, Kessel GJT, van der Werf W (2009) Scenario approach for assessing the utility of dispersal information in decision support for aerially spread plant pathogens, applied to Phytophthora infestans. Phytopathology 99: 887–895.

21. Rossi V, Caffi T, Giosuè S, Bugiani R (2008). A mechanistic model simulating primary infections of downy mildew in grapevine. Ecol. Modell 212: 480–491.

22. Aylor DE, Schmale III DG, Shields EJ, Newcomb M, Nappo CJ (2011). Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agric. For. Meteorol 151: 251–260.

23. Ford A (2009) Modeling the Environment, second ed. Island Press, Washington, DC, USA. 380 pp.

24. Powlesland R (1953) On the biology of *Bremia lactucae*. Transactions of the British Mycological Society. 37: 362± 371.

25. Norwood JM, Crute IR (1985) Further characterization of field resistance in lettuce to *Bremia lactucae* (downy mildew). Plant Pathol 34: 481–486.

26. Bhaskara Reddy MV, Kushalappa AC, Stephenson MMP (1996). Effect of solar radiation on the survival of Bremia lactucae spores on lettuce. Phytoprotection 77: 137.

27. Kranz J (1974) Epidemics of Plant Diseases – Mathematical Analysis and Modeling (Ecological Studies, Vol. 13). 170 S., 46 Abb., 12 Tab. Berlin-Heidelberg-New York. Springer-Verlag. DM 59,80.

28. Piñeiro G, Perelman S, Guerschman JP, Paruelo JM (2008) How to evaluate models: Observed vs. predicted or predicted vs. observed? Ecol. Modell. 216: 316–322.

29. Nieto H, Aguado I, Chuvieco E, Sandholt I (2010) Dead fuel moisture estimation with MSG–SEVIRI data. Retrieval of meteorological data for the calculation of the equilibrium moisture content. Agric. For. Meteorol 150: 861–870.

30. Smith EP, Rose KA (1995) Model goodness-of-fit analysis using regression and related techniques. Ecol. Modell 77: 49–64.

31. Olanya OM, Honeycutt CW, Tschöepe B, Kleinhenz B, Lambert DH, Johnson SB (2012) Effectiveness of SIMBLIGHT1 and SIMPHYT1 models for predicting

Phytophthora infestans in north-eastern United States. Arch. Phytopathol. Plant Prot. 45: 1558–1569.

32. Bugiani R, Govoni P, Bottazzi R, Giannico P, Montini B, Pozza M (1995). Monitoring airborne concentrations of sporangia of Phytophthora infestans in relation to tomato late blight in Emilia Romagna, Italy. Aerobiologia. 11: 41–46.

33. Hadders J (2008). An example of integrated forecasting system for Phytophthora infestans on potato. In: Ciancio, A., Mukerji, K.G. (Eds.), Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria. Integrated Management of Plant Pests and Diseases 3: 179–189.

5.8. Supporting Information

All STELLA equations used in the model are given below.

RNSC(t) = RNSC(t - dt) + (Surviving – Deposited- UDC) * dt
INIT RNSC = 0
INFLOWS:
Surviving = RNEC*Surv_rate
OUTFLOWS:
UDC= 29/30*RNSC
Deposited = 1/30*RNSC
IPLAD(t) = IPLAD(t - dt) + (Being_infected) * dt
INIT IPLAD = 0
INFLOWS:
Being_infected = ((ACC*10*1/3)/LAI)*0.0135*0.0001
Number_of_lesion(t) = Number_of_lesion(t - dt) + (Incubation) * dt
INIT Number_of_lesion = 0

INFLOWS:

Incubation = CONVEYOR OUTFLOW

RNDC(t) = RNDC(t - dt) + (Deposited) * dt

INIT RNDC = 0

INFLOWS:

Deposited = 1/30*RNSC

RNEC(t) = RNEC(t - dt) + (Escaped - Surviving - death) * dt

INIT RNEC = 0

INFLOWS:

Escaped = RNRC*Escp_rate

OUTFLOWS:

Surviving = RNEC*Surv_rate

death = (1-Surv_rate)*RNEC

RNRC(t) = RNRC(t - dt) + (Released - Escaped - UEC) * dt

INIT RNRC = 0

INFLOWS:

Released = Sp*PSL

OUTFLOWS:

Escaped = RNRC*Escp_rate

UEC = 1-Escp_rate

PSL(t) = PSL(t - dt)

INIT PSL = IF(5< Tp<=25) AND (RH>=85) THEN 5000 ELSE 0

TRANSIT TIME = 1

CAPACITY = INF

INFLOW LIMIT = INF

RNGC(t) = RNGC(t - dt) + (Germinated - Incubation) * dt

INIT RNGC = 0

TRANSIT TIME = 240

CAPACITY = INF

INFLOW LIMIT = INF

INFLOWS:

Germinated = RNDC*Germ

OUTFLOWS:

Incubation = CONVEYOR OUTFLOW

ACC = GRAPH(TIME)

(6.00, 5.00), (6.12, 5.00), (6.23, 5.00), (6.35, 5.00), (6.46, 5.00), (6.58, 5.00), (6.70, 5.00), (6.81, 5.00), (6.93, 5.00), (7.04, 5.00), (7.16, 5.00), (7.28, 5.00), (7.39, 5.00), (7.51, 5.00), (7.62, 5.00), (7.74, 5.00), (7.86, 5.00), (7.97, 5.00), (8.09, 5.00), (8.20, 5.00), (8.32, 5.00), (8.43, 5.00), (8.55, 5.00), (8.67, 5.00), (8.78, 5.00), (8.90, 5.00), (9.01, 5.00), (9.13, 5.00), (9.25, 5.00), (9.36, 5.00), (9.48, 5.00), (9.59, 5.00), (9.71, 5.00), (9.83, 5.00), (9.94, 5.00), (10.1, 5.00), (10.2, 5.00), (10.3, 5.50), (10.4, 6.00), (10.5, 6.50), (10.6, 7.00), (10.8, 7.50), (10.9, 8.00), (11.0, 8.33), (11.1, 8.67), (11.2, 9.00), (11.3, 9.33), (11.4, 9.67), (11.6, 10.0), (11.7, 10.8), (11.8, 11.7), (11.9, 12.5), (12.0, 13.3), (12.1, 14.2), (12.3, 15.0), (12.4, 15.8), (12.5, 16.7), (12.6, 17.5), (12.7, 18.3), (12.8, 19.2), (13.0, 20.0), (13.1, 20.7), (13.2, 21.3), (13.3, 22.0), (13.4, 22.7), (13.5, 23.3), (13.7, 24.0), (13.8, 24.2), (13.9, 24.3), (14.0, 24.5), (14.1, 24.7), (14.2, 24.8), (14.3, 25.0), (14.5, 23.8), (14.6, 22.7), (14.7, 21.5), (14.8, 20.3), (14.9, 19.2), (15.0, 18.0), (15.2, 17.3), (15.3, 16.7), (15.4, 16.0), (15.5, 15.3), (15.6, 14.7), (15.7, 14.0), (15.9, 13.7), (16.0, 13.3), (16.1, 13.0), (16.2, 12.7), (16.3, 12.3), (16.4, 12.0), (16.6, 11.7), (16.7, 11.3), (16.8, 11.0), (16.9, 10.7), (17.0, 10.3), (17.1, 10.0), (17.2, 10.0), (17.4, 10.0), (17.5, 10.0), (17.6, 10.0), (17.7, 10.0), (17.8, 10.0), (17.9, 9.67), (18.1, 9.33), (18.2, 9.00), (18.3, 8.67), (18.4, 8.33), (18.5, 8.00), (18.6, 7.50), (18.8, 7.00), (18.9, 6.50), (19.0, 6.00), (19.1, 5.50), (19.2, 5.00), (19.3, 4.67), (19.4, 4.33), (19.6, 4.00), (19.7, 3.67), (19.8, 3.33), (19.9, 3.00), (20.0, 2.83), (20.1, 2.67), (20.3, 2.50), (20.4, 2.33), (20.5, 2.17), (20.6, 2.00), (20.7, 1.67), (20.8, 1.33), (21.0, 1.00), (21.1, 0.667), (21.2, 0.333), (21.3, 0.00), (21.4, 0.00), (21.5, 0.00), (21.7, 0.00), (21.8, 0.00), (21.9, 0.00), (22.0, 0.00)

Asymp1 = 0.966-0.000051*(Tp)^3 + (-15.575/(Tp)^2)

Asymp2 = 0.385 +0.054*Tp -0.0024*(Tp)^2

Escp_rate = 0.073*Ws-0.0087

Germ = Asymp2*EXP(-EXP(-r2*(LWD-2)))

LAI = 3

LWD = GRAPH(TIME)

(0.00, 3.00), (1.00, 4.00), (2.00, 5.00), (3.00, 6.00), (4.00, 7.00), (5.00, 8.00), (6.00, 9.00), (7.00, 10.0), (8.00, 11.0), (9.00, 12.0), (10.0, 0.00), (11.0, 0.00), (12.0, 0.00), (13.0, 0.00), (14.0, 1.00), (15.0, 0.00), (16.0, 0.00), (17.0, 0.00), (18.0, 0.00), (19.0, 1.00), (20.0, 2.00), (21.0, 3.00), (22.0, 4.00), (23.0, 2.00)

r1 = 0.5

r2 = -1.154 +0.327*Tp -0.011*(Tp)^2

reduction_rate = -16.43 +4.59*Tp

RH = 85

Sp = Asymp1*(1+25118.86*exp(-r1*LWD))^(-0.909)

SR = GRAPH(TIME)

(0.00, 0.00), (0.167, 0.00), (0.333, 0.00), (0.5, 0.00), (0.667, 0.00), (0.833, 0.00), (1.00, 0.00), (1.17, 0.00), (1.33, 0.00), (1.50, 0.00), (1.67, 0.00), (1.83, 0.00), (2.00, 0.00), (2.17, 0.00), (2.33, 0.00), (2.50, 0.00), (2.67, 0.00), (2.83, 0.00), (3.00, 0.00), (3.17, 0.00), (3.33, 0.00), (3.50, 0.00), (3.67, 0.00), (3.83, 0.00), (4.00, 0.00), (4.17, 0.00), (4.33, 0.00), (4.50, 0.00), (4.67, 0.00), (4.83, 0.00), (5.00, 0.00), (5.17, 0.00), (5.33, 0.00), (5.50, 0.00), (5.67, 0.00), (5.83, 0.00), (6.00, 0.00), (6.17, 0.00), (6.33, 0.00), (6.50, 0.00), (6.67, 0.00), (6.83, 0.00), (7.00, 0.00), (7.17, 0.00), (7.33, 0.00), (7.50, 0.00), (7.67, 0.00), (7.83, 0.00), (8.00, 0.00), (8.17, 0.00), (8.33, 0.00), (8.50, 0.00), (8.67, 0.00), (8.83, 0.00), (9.00, 0.00), (9.17, 0.00), (9.33, 0.00), (9.50, 0.00), (9.67, 0.00), (9.83, 0.00), (10.0, 0.00), (10.2, 0.00), (10.3, 0.00), (10.5, 0.00), (10.7, 0.00), (10.8, 0.00), (11.0, 0.00), (11.2, 0.00), (11.3, 0.00), (11.5, 0.00), (11.7, 0.00), (11.8, 0.00), (12.0, 0.00), (12.2, 0.00), (12.3, 0.00), (12.5, 0.00), (12.7, 0.00), (12.8, 0.00), (13.0, 0.00), (13.2, 0.00), (13.3, 0.00), (13.5, 0.00), (13.7, 0.00), (13.8, 0.00), (14.0, 0.00), (14.2, 0.00), (14.3, 0.00), (14.5, 0.00), (14.7, 0.00), (14.8, 0.00), (15.0, 0.00), (15.2, 0.00), (15.3, 0.00), (15.5, 0.00), (15.7, 0.00), (15.8, 0.00), (16.0, 0.00), (16.2, 0.00), (16.3, 0.00), (16.5, 0.00), (16.7, 0.00), (16.8, 0.00), (17.0, 0.00), (17.2, 0.00), (17.3, 0.00), (17.5, 0.00), (17.7, 0.00), (17.8, 0.00), (18.0, 0.00), (18.2, 0.00),

(18.3, 0.00), (18.5, 0.00), (18.7, 0.00), (18.8, 0.00), (19.0, 0.00), (19.2, 0.00), (19.3, 0.00), (19.5, 0.00), (19.7, 0.00), (19.8, 0.00), (20.0, 0.00), (20.2, 0.00), (20.3, 0.00), (20.5, 0.00), (20.7, 0.00), (20.8, 0.00), (21.0, 0.00), (21.2, 0.00), (21.3, 0.00), (21.5, 0.00), (21.7, 0.00), (21.8, 0.00), (22.0, 0.00), (22.2, 0.00), (22.3, 0.00), (22.5, 0.00), (22.7, 0.00), (22.8, 0.00), (23.0, 0.00)

Surv_rate = IF SR<1 THEN 0.8 ELSE 0.6

Tp = GRAPH(TIME)

(0.00, 15.9), (1.00, 16.3), (2.00, 14.8), (3.00, 13.8), (4.00, 13.6), (5.00, 13.1), (6.00, 12.6), (7.00, 14.1), (8.00, 17.5), (9.00, 19.4), (10.0, 21.3), (11.0, 22.1), (12.0, 22.3), (13.0, 20.2), (14.0, 15.2), (15.0, 18.3), (16.0, 20.9), (17.0, 20.6), (18.0, 20.4), (19.0, 17.8), (20.0, 16.3), (21.0, 15.1), (22.0, 14.2), (23.0, 16.4)

Ws = GRAPH(TIME)

(0.00, 0.00), (1.00, 1.12), (2.00, 0.00), (3.00, 0.00), (4.00, 0.00), (5.00, 0.00), (6.00, 0.00), (7.00, 0.00), (8.00, 1.68), (9.00, 1.68), (10.0, 1.12), (11.0, 3.92), (12.0, 3.08), (13.0, 2.52), (14.0, 1.68), (15.0, 3.92), (16.0, 0.84), (17.0, 2.52), (18.0, 1.12), (19.0, 0.00), (20.0, 0.00), (21.0, 0.00), (22.0, 0.00), (23.0, 0.00)

CHAPITRE 6

DISCUSSION ET CONCLUSION GÉNÉRALE

6.1. Les problématiques et résultats de recherche

Ce projet de doctorat voulait répondre spécifiquement à trois questions à savoir: quelle est la dynamique et la distribution de l'inoculum des agents pathogènes du mildiou de la pomme de terre et de la laitue? Quel lien existe-il entre la concentration aérienne d'inoculum et le développement du mildiou? Si ce lien est significatif, est-il possible de prédire les concentrations aériennes d'inoculum?

L'étude de la dynamique de l'inoculum aérien a permis de confirmer la nécessité d'intégrer le suivi en temps réel des spores dans les modèles de prévision à défaut de ne pas pouvoir prédire la quantité d'inoculum aérien (Fall et al., 2015a, Fall et al., 2015b). Cette étude a permis de mettre en évidence les avantages et limites de l'utilisation d'un réseau de capteur de spores afin de pondérer les risques estimés par les modèles de prévision et d'évaluer graduellement l'efficacité des stratégies de gestion. Cette étude est aussi une première à avoir suggéré l'utilisation de l'aire sous la courbe de progression de l'inoculum comme moyen d'évaluer la progression du mildiou et ainsi identifier les zones de forte progression du mildiou de la pomme de terre. Ces solutions sont aujourd'hui adoptées dans les zones de production de la pomme de terre du Canada et dans la production de laitue au Québec afin de rationaliser l'usage des fongicides.

L'étude sur l'efficience contaminatrice, définie comme le rapport entre la concentration aérienne de spores et l'intensité du mildiou, a permis d'établir des seuils d'intervention basés sur la concentration aérienne des spores (Fall et al., 2015b; Fall et al., 2015c). Dans le pathosystème de la pomme de terre, le seuil d'intervention varie en fonction des lignées clonales de *P. infestans* (Fall et al., 2015c). En effet, cette étude a montré que certaines des lignées clonales de *P. infestans* sont plus agressives que d'autres. Dans le pathosystème de la laitue, le seuil d'intervention n'a pas été défini en fonction des lignées clonales puisque celles-ci n'ont pas encore été caractérisées au Canada (Fall et al., 2015b). Cette étude a permis, entre autres, de montrer que sous nos conditions climatiques deux heures de mouillure sont suffisantes pour l'établissement d'une infection par *B. lactucae* contrairement aux trois à quatre heures de mouillure proposées dans la littérature (Scherm et van Bruggen, 1994). De plus, considérer la mouillure matinale et la mouillure de fin de journée des feuilles de laitue permettrait d'améliorer les prédictions du risque d'infection des modèles prévisionnels (Fall et al., 2015b). La fiabilité et la pertinence d'un réseau de capteurs de spores dépendent de la précision dans le comptage des spores. Ce comptage se fait habituellement à l'aide d'un microscope, ce qui demande beaucoup de temps et de l'expertise. Dans le pathosystème de la pomme de terre, un outil moléculaire pour la détection des spores de *Phytophthora infestans* a été développé pour minimiser les erreurs de comptage et pour rendre l'information rapidement disponible aux producteurs (Fall et al. 2015c), un tel outil est à développer dans le pathosystème de la laitue.

La simulation dynamique a permis de simuler et de quantifier les concentrations aériennes de spores de *Bremia lactucae* (Fall et al. 2015, soumis dans PlosOne). Les résultats montrent que les concentrations horaires de spores prédites par le modèle suivent bien celles mesurées avec les capteurs de spores. La relation entre le nombre de spores observées à l'heure et le nombre de spores prédit à l'heure est linéaire. Dans plus de 94% des cas les coefficients de détermination de la régression

entre le nombre de spores observés et celui prédit sont supérieurs à 0,7. Cette relation linéaire s'observe aussi entre les concentrations journalières de spores observées et celles prédites par le modèle. C'est une première qu'un modèle quantitatif de simulation des concentrations aériennes de spores soit publié dans la littérature scientifique en épidémiologie végétale, par ailleurs, ce modèle représente une avancée significative dans l'aérobiologie de *B. lactucae*. Cependant, il est à améliorer notamment dans l'optique de pouvoir développer un modèle générique comme outil de recherche pour l'étude de l'aérobiologie des oomycètes phytopathogènes.

Les résultats obtenus durant ce projet de doctorat sont conformes aux objectifs fixés. En effet, les résultats obtenus ont participé à l'amélioration des connaissances sur l'aérobiologie de *P. infestans* et celle de *B. lactucae*. Les étapes à venir seront d'incorporer le modèle développé dans ce projet comme module dans un système de support décisionnel pour prédire efficacement les symptômes de mildiou. Les auteurs espèrent que ce modèle constituera une base pour le développement d'un modèle générique pour tous les oomycètes phytopathogènes à dispersion aérienne.

6.2. Conclusion et perspectives de recherche

Ce projet de doctorat s'inscrivait dans une optique d'amélioration des connaissances sur l'aérobiologie des oomycètes notamment celle de *Phytophthora infestans* et *Bremia lactucae*. Il a permis des avancées à plusieurs niveaux :

Sur le plan scientifique, ce projet a permis d'établir une nouvelle compréhension de la dynamique spatiotemporelle du mildiou. Il se situe entre le développement de

connaissance et le développement d'une application, par ailleurs, il se classerait au niveau de la recherche fondamentale inspirée par une application. De plus, ce projet représente une bonne introduction sur l'aérobiologie comparative des oomycètes, en comparant la dynamique de l'inoculum aérien de *P. infestans* et celle de l'inoculum aérien de *B. lactucae*. En ce sens, il a permis d'établir des prémices pour le développement d'un modèle générique de simulation de la dynamique des spores d'oomycètes phytopathogènes à dispersion aérienne.

Sur le plan personnel, ce projet m'a permis d'atteindre trois objectifs connexes :

- Maîtriser la démarche scientifique et les compétences nécessaires pour mener une recherche à terme afin d'être apte à assumer la responsabilité d'activités de recherche;
- Développer des compétences en phytopathologie, en épidémiologie en modélisation et en statistiques;
- Améliorer mes compétences en communication et en rédaction scientifique.

Du point de vue agricole, ce projet présente un fort potentiel d'application commerciale. En effet, l'outil d'aide à la décision développé pourra contribuer à une meilleure rationalisation de la régie d'application des fongicides dans la culture de pommes de terre et de laitue. Les auteurs espérent que les résultats obtenus participeront à réduire substantiellement l'utilisation de fongicides sans compromettre le rendement agricole.

Ce projet de doctorat a aussi mis en évidence un certain nombre de manquements dans nos connaissances qui pourront faire l'objet de futures études. Entre autres, le nombre de capteurs de spores par unité de surface dans une région

géographique donnée est à déterminer. Cette information est impérative pour l'utilisation de l'information sur la dynamique des spores à l'échelle d'une région de production. La caractérisation des lignées clonales de *B. lactucae* présentes au Canada est aussi nécessaire puisqu'à l'image des lignées clonales de *P. infestans*, les lignées clonales de *B. lactucae* devraient présenter différents niveaux d'agressivité. Aussi, un des défis dans la gestion des mildious est d'identifier les sources d'inoculum primaire. Les oospores et les hôtes alternatifs sont identifiés dans la littérature comme des sources potentielles d'inoculum primaire (Michelmore et al., 1988; Olanya et al., 2009; Lebeda et al., 2002). Cependant, aussi bien dans le pathosystème de la pomme de terre que dans celui de la laitue, il n'y a pas d'évidence claire dans la littérature sur la survie des oospores durant l'hiver au Canada. Ainsi, des études sur la survie des oospores durant l'hiver et le rôle des hôtes alternatifs comme source d'inoculum est nécessaire.

En suggérant un outil de gestion et de suivi des agents pathogènes à dispersion aérienne, ce projet de doctorat contribue au développement d'un réseau de biovigilance dans l'optique de soutenir la production agricole face aux aléas des changements climatiques. En effet, le rapport du groupe international d'étude sur le climat (GIEC) de 2014, prévoit, entre autres, un développement accru des infections fongiques et propose le renforcement de l'épidémiosurveillance (Edenhofer et al., 201 4).

BIBLIOGRAPHIE

Aylor, D.E. (1993). Relative collection efficiency of Rotorod and Burkard spore samplers for airborne Venturia inaequalis ascospores. Phytopathology, 83, 1116–1119.

Aylor, D.E., Fry, W.E., Mayton, H., et Andrade-Piedra, J.L. (2001). Quantifying the rate of release and escape of Phytophthora infestans sporangia from a potato canopy. Phytopathology, 91, 1189–1196.

Aylor, D.E. (2003). Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. Ecology, 84, 1989–1997.

Aylor, D.E., Schmale III, D.G., Shields, E.J., Newcomb, M., et Nappo, C.J. (2010). Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agricultural and Forest Meteorology, 151, 251–260.

American society of microbiology (ASM) (2009). Fives questions about Oomycetes. In American society of microbiology. http://schaechter.asmblog.org/schaechter/2009/11/five-1.html

Andreu A., B., Caldiz D. O. (2005). Early management of late blight (Phytophthora infestans) by using systemic fungicides applied to seed-potato tubers. Crop protection, vol. 25, 281-286.

Bugiani, R., Govonie, P., Bottazzi R., Giannico, P., Montini, B., et Pozza, M. (1994). Monitoring airborne concentration of sporangia of Phytophthora infestans in relation to tomato late blight in Emilia Romagna, Italy. Aerobiologia, 11, 41–46.

Blancard D. (2011). Biologie, épidémiologie. In Institut national de la recherche agronomique (INRA). http://ephytia.inra.fr/salade/salade_utilisateur/index_appli.php?portail=legumes&produit=salade&main=2&ssrub1=19&ssrub2=18&ssrub3=35&id_fiche=12&theme=35

Blum M., Gamper H., A., Waldner M., Sierotzki H. et Gisi, U. (2011). The cellulose synthase 3 (Ces A3) gene of oomycetes: structure, phylogeny and influence on sensitivity to carboxylic acide amide (GAA) fungicides. Bristish mycological society. Fungal biology. Vol. 116, 529-542.

Chamont, S. (2010). Oomycota. In Institut national de la recherché agronomique (INRA). http://ephytia.inra.fr/hypp/hypp_utilisateur/index_appli.php?portail=bioagresseurs&produit=hypp&main=3&ssrub1=7&ssrub2=56

Cordier T. (2007). Recherche d'Oomycètes dans des peuplements forestiers d'épérissants en Pologne. École d'ingénieurs HES-SO de Lulier (Suisse). Document produit en vue de l'obtention du grade d'ingénieur en agronomie. 79 pages.

Carisse O., Philion V. (2002). Meteorological factors affecting periodicity and concentration of airborne spores of Bremia lactucae. Canadian Journal of plant pathology, vol. 24, 184-193.

Carlo G., Xavier G. (2008). Modélisation et statistique spatiales. Mathématiques et applications vol. 63 pp 253.

Day, J.P., Kell, D.B., et Griffith, G.W. (2001). Differentiation of Phytophthora infestans sporangia from other airborne biological particles by flow cytometry. Applied and Environmental Microbiology, 68, 37–45.

Department of Plant Pathology of Cornell University (DPPCU) (s.d). Overview of Oomycetes. In Cornell University. http://www.plantpath.cornell.edu/Courses/pp309/Lectures/Lecture10.pdf

Departement of biology of Aberystwyth University (DBAU) (s.d). Fungal biology. In Aberystwyth University. http://www.aber.ac.uk/fungi/fungi/taxonomy.htm#

Edenhofer, O., Pichs-Madruga, R., Sokona, Y., Minx, J.C., Farahani, E., Kadner, S., Seyboth, K., et al. (2014).Mitigation of climate change. Working group III. Intergouvernmental panel on climate change. 1435 pages. http://www.ipcc.ch/pdf/assessment-report/ar5/wg3/ipcc_wg3_ar5_full.pdf

Fry, W.E., et Goodwin, S.B. (1997). Resurgence of the Irish potato famine fungus. BioScience, 47, 363–371.

Fry, W., E. et Niklaus J., G. (2010). Introduction to oomycetes. In American phytopathological society (APSnet). http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/IntroOomycetes.aspx

Forest Phytophthora of the World (FPW) (2012). Glossary. In forest Phytophthora of the world. http://forestphytophthoras.org/glossary/

Fall, M. L., Van der Heyden, H., Brodeur, L., Leclerc, Y., Moreau, G., and Carisse, O. (2015a). Spatiotemporal variation in airborne sporangia of Phytophthora infestans: Characterization and initiative toward improving potato late blight risk estimation. Plant Pathol. 64:178-190.

Fall, M. L., Van der Heyden, Beaulieu, C., and Carisse, O. (2014b). Bremia Lactucae Infection Efficiency in Lettuce is Modulated by Temperature and Leaf Wetness

Duration under Quebec Field Conditions. Plant Disease. In press. doi:10.1094/PDIS-05-14-0548.

Fall ML, Tremblay DM, Gobeil-Richard M,Couillard J, Rocheleau H, Van der Heyden H, et al.(2015c) Infection Efficiency of Four Phytophthora infestans Clonal Lineages and DNA-Based Quantification of Sporangia. PLoS ONE 10(8):e0136312. doi:10.1371/journal.pone.0136312.

Flier WG, Turkensteen LJ, 1999. Foliar aggressiveness of Phytophthora infestans in three potato growing regions in the Netherlands. European Journal of Plant Pathology 105, 381–388.

Grenville-Briggs L., J., Anderson V., L., Fugelstad J., Avrova A., O., Bouzenzana J., Williams A., Wawra S., Whisson S., C., Birch P., R., Bulone V. and West P. (2008). Cellulose synthsesis in Phytophthora infestans is required for normal appressorium formation and successful infection of potato. Plant cell, vol. 20, 720-738.

Gosme M. (2007). Comment analyser la structure spatiale et modéliser le développement spatio-temporel des épiphyties? Synthèse. Revue canadienne de phytopathologie, vol. 30, 4-23.

Henderson, D., Williams, C.J., et Miller, J.S. (2007). Forecasting late blight in potato crops of southern Idaho using logistic regression analysis. Plant Disease, 91, 951–956.

Hurst C., J., Crawford R., L., Garland J., L., Lipson D., A., Mills A., L., Stetzenbach L., D. (2007). Environmental microbiology. Manual of environmental microbiology. Third edition. American society of microbiology Press. 1293 pages.

Haldar, K., Kamoun, S., Hiller, N. L., Bhattacharje S and van Ooij, C. (2006) Common infection strategies of pathogenic eukaryotes. Nature Reviews Microbiology 4, 922-931.

Hwang S., C., Ko W., H. (1977). Biology of chlasmydospores, sporangia, and zoospores of Phytophthota cinnamomi in soil. Phytopathology 68: 726-731.

Institut National de la Recherche Agronomique (INRA) (2010). Bremia lactucae Regel, Mildiou. In Institut national de la recherché agronomique. http://ephytia.inra.fr/salade/salade_utilisateur/index_appli.php?portail=legumes&produit=salade&main=2&ssrub1=19&ssrub2=18&ssrub3=35&id_fiche=12

Judelson, H. S. and Blanco, F. A. (2005) The spores of Phytophthora: weapons of the plant destroyer. Nature Reviews Microbiology 3, 47-58.

Jeffrey, S.I.B., Jinks, J.L., Grindle, M., 1962. Interracial variation in Phytophthora infestans and field resistance to potato blight. Genetica 32, 323-338.

Johnson DA, 1980. Effect of low temperature on the latent period of slow and fast rusting winter wheat genotypes. Plant Disease 64, 1006-1008.

Kirk W., Wharton P., Hammerschmidt R., Samen F., A., Douches D. (2004). Michigan potato diseases: late blight. Extension Bulletin E-2945.1-8.

Kushalappa A., C. (2001). Development of system to forecast risk levels of downy mildew on lettuce (Bremia lactucae). International journal of pest management, vol. 47, 1-5.

Kranz J., and Rotem J. (1988). Experimental techniques in plant disease epidemiology. Springer-Verlag. 299 pages

Leonard, R., Dowley, L.J., Rice, B., et Ward, S. (2001). Comparison of the NegFry decision support system fungicide application for the control of potato late blight in Ireland. Potato Research, 44, 327–336.

Lamour K., Kamoun S. (2009). Oomycete genetics and genomics, diversity, interaction and research tools. Willey-Blackwell. 574 pages.
Lepoivre P. (2003). Phytopathologie, bases moléculaires et biologiques des pathosystèmes et fondements des stratégies de lutte. De Boeck. 427 pages.

Lebeda A, Pink DAC, Astley D.(2002). Aspects of the interactions between wild Lactuca spp. and related genera and lettuce downy mildew (Bremia lactucae). In: Spencer-Phillips PTN, Gisi U, Lebeda A, eds. Advances in Downy Mildew Research. Dordrecht, the Netherlands: Kluwer Academic,85–117.

Lee SJ, Rose JKC. (2010). Mediation of the transition from biotrophy to necrotrophy in hemibiotrophic plant pathogens by secreted effector proteins. Plant Signal Behav 5: 769–772.

Madden, L.V., Hughes, G., et Van den Bosch, F. (2007). The study of plant disease epidemics. The American Phytopathological Society, St. Paul, MN. 421 p.

Madoui M-A. (2009). Identification d'effecteurs du pouvoir pathogène et de voies métaboliques chez l'oomycéte Aphanomyces euteiches par une approche génomique. Thèse de doctorat de l'Université de Toulouse III, Bioscience Végétale. 125 pages.

Marks G., E. (1965). The cytology of Phytophthora infestans. Chromosoma (Berl.), vol. 16, 681-692.

Montarry J. (2007). Réponse adaptative des populations de Phytophthora infestans, agent du mildiou de la pomme de terre, au déploiement en culture de son hôte Salanum tuberosum. École nationale supérieure agronomique de Rennes (France). Thèse de doctorat.

Michelmore, R.W., llott, T., Hulbert, S.H., and Farrara, B. (1988). The downy mildews. Advances in Plant Pathology 6: 53-79.
Mehta YR, Zadoks JC, 1970. Uredospore production and sporulation period of Puccinia recondita f.sp. triticina on primary leaves of wheat. Netherlands Journal of Plant Pathology 76, 267–276.

Nyankanga R., O., Olanya O., M., Ojiambo P.S., Wien H., C., Honeycutt C. W., et Kirk W., W. (2010). Validation of tuber blight (Phytophthora infestans) prediction model. Crop protection, vol. 30, 547-553.

Noll, K.E. (1970). A rotary inertial impactor for sampling giant particles in the atmosphere. Atmospheric Environment, 4, 9–19.

National center for biotechnology information (NCBI)(s.d). In National center for biotechnologyinformation. http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?name=stramenopiles

Olanya, O. M., Plant, A. B., Larkin, R. P., and Honeycutt, C., W. (2009). Infection potential of hairy nightshade (solanum sarrachoides) by Phytophthora infestans and late blight implications of the alternate host. J Phytopathol 157:427-437.

Parker J. (2009). Molecular aspects of plant disease resistance. Annuel plant reviews, volumes 34, Willey-Blackwell 385 pages.

Platt B. (2008). Maladies de la pomme de terre causes par des Oomycètes. Cahiers agricultures vol.17, n° 4. 361-367.

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carlier, J., Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. Plant Pathology, 58, 409-424.

Ristaino, J.B. (2002). Tracking migrations of the Irish potato famine pathogen, *Phytophthora infestans*. *Microbes and Infection*, *4*, 1369–1377.

Rapilly F. (1991). L'épidémiologie en pathologie végétale : mycoses aériennes. Institut national de la recherche agronomique. 317 pages.

Roger A., C., J., Salam M., U., Maling T., J., Diggle A., J., Thackray D., J. (2010). Principles of predicting plant virus disease epidemics. Annu. Rev. Phytopathol., vol. 48, 179-203.

Skelsey, P., Kessel, G.J.T., Holtlag, A.A.M., Moene, A.F., et Van der Werf, W. (2008a). Regional spore dispersal as a factor in disease risk warnings for potato late blight: A proof of concept. *Agricultural and Forest Meteorology*, *149*, 419–430.

Skelsey, P., Holtlag, A.A.M., et Van der Werf, W. (2008b). Development and validation of quasi-Gaussian plume model for transport of botanical spores. *Agricultural and Forest Meteorology*, *148*, 1383–1394.

Skelsey, P., Kessel, G.J.T., Rossing, W.A.H., et Van der Werf, W. (2008c). Parameterization and evaluation of a spatiotemporal model of the potato late blight pathosystem. *Phytopathology*, *99*, 290–300.

Skelsey, P. (2008d). Multi-scale modeling of potato late blight epidemics. Ph.D. thesis, Wageningen University. 257 p.

Skelsey, P., Rossing, W.A.H., Kessel, G.J.T., et Van der Werf, W. (2009). Scenario approach for assessing the utility of dispersal information in decision support for aerially spread plant pathogens, applied to *Phytophthora infestans*. *Phytopathology*, *99*, 887–895.

Schuman G.L., D'Arcy C.J. (2005). Late blight of potato and tomato. In American phytopathological society (APS net). http://www.apsnet.org/edcenter/intropp/lessons/fungi/Oomycetes/Pages/LateBlight.aspx

Su H., van Bruggen A., H., C., Subbarao K., V. (1999). Spore release of *Bremia* lactucae on lettuce is affected by timing of light initiation and decrease in relative humidity. Phytopathology vol. 90, 67-71.

Samalens J-C. (2009). Stratégies d'échantillonnage des dommages forestiers à l'échelle du paysage. Thèse de doctorat, Université de Bordeaux 1, science et environnement, 245 pages.

Scherm, H., and van Bruggen, A.H.C. (1994). Weather variables associated with infection of lettuce by downy mildew (Bremia lactucae) in coastal California. Phytopathology 84:860-865.

Taylor, L.R. (1961). Aggregation, variance and mean. Nature, 189, 732–735.

Van der Plank, J. E. (1963). Plant diseases: Epidemics and control. New York & London, Academic Press 349 pages.

World agrometeorological information service (WAMIS) (s.d.). Aerobiology. In World agrometeorological information service. http://www.wamis.org/agm/gamp/GAMP_Chap15.pdf