Polyetic development of the obligate biotrophic plant pathogen *Peronospora destructor*

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"All models are approximations. Essentially, all models are wrong, but some are useful. However, the approximate nature of the model must always be borne in mind."

George E. P. Box

Table of content

List of figures

General introduction

Litterature review

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Supplementary figures for Chapter 6

List of tables

Litterature review

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Supplementary tables for Chapter 6

List of abbreviations

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ATH	Mean temperature during harvest (°C)
CABI	Commonwealth Agricultural Bureau
CLO	Ste-Clothilde-de-Chateauguay
DAPC	Discriminant analysis of principal components
DC	Number of DownCast sporulation periods
DSI	Disease intensity (from 0 to 3)
DSP	Number of morning precipitation events (between 7:00 and 12:00)
DS_PY	Final RDI previous year
DS_P2Y	Final RDI the year before previous year
GBS	Genotyping by sequencing
HL	Hellmann number (sum of average daily temperatures below 0 °C)
HMS	Highest mean of success
HT4_24IP.	Number of hours with temperature between 4 °C and 24 °C (between 6:00 and 20:00)
HT4_24N	Number of hours with temperature between 4 °C and 24 °C (between 20:00 and 6:00)
HTS	High throughput sequencing
IBIS	Institute of Integrative Biology and Systems
IC	Internal control
ITS	Internal transcribed spacer region
LAMP-PCR	Loop isothermal mediated amplification
LD	Linkage disequilibrium
MLG	Multilocus genotype
MTH	Minimum temperature at harvest (°C)
NAP	Napierville
NHDT_28	Number of hours with temperatures above 28 °C (between 6:00 and 20:00)
NHNT_28	Number of hours with temperatures above 28 °C (between 20:00 and 6:00)
NHR_90	Number of hours with relative humidity above 90%
NREIP	Number of precipitation events during infection period
NSP	Number of night precipitation events (between 24:00 and 6:00)

ODM	Onion downy mildew
OSRF	Off growing season rainfall (mm)
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
RAD	Average solar radiation (J m^{-2})
RAPD	Random amplified polymorphic DNA markers
RDI	Regional disease incidence (% of infected fields)
RH	Relative humidity
RMSE	Root mean square error
RPA-PCR	Recombinase polymerase amplification
SCAR	Sequence-characterized amplified region markers
SCOV	Snow cover (mm)
SE	Standard error
SHERR	Sherrington
SNP	Single-nucleotide polymorphism
Tm	Primers melting temperature
TRH	Total rainfall at harvest (mm)
UPGMA	Underweight paired group method with arithmetic mean
VCF	Variant Calling Format

Abstract

Onion downy mildew, caused by *Peronospora destructor*, is a serious threat for onion growers worldwide. In southwestern Québec, Canada, a steady increase in the occurrence of onion downy mildew has been observed since the mid-2000s, reaching 33% of affected onion fields in 2014. On onion, *P. destructor* can develop local and systemic infections producing numerous sporangia, which act as initial inoculum locally and for neighboring areas. Several *Peronospora* species are carried by wind over short and long distances, from warmer climates where they survive on living plants to cooler climates. In eastern Canada, this annual flow of sporangia was thought to be the main source of *P. destructor*. However, it also produces oospores capable of surviving in soils and tissues for a prolonged period. In this thesis, it was hypothesized that the impact of climate change in southwestern Quebec, with warmer temperatures in the fall and milder winters, favored the survival of overwintering inoculum.

The first sub-objective aimed to investigate the variations in ODM incidence and epidemic onset and identify the meteorological variables that influence its polyetic development (i.e., over multiple seasons), using observational data collected over 31 consecutive years. This analysis showed that the first disease observation date, 10% epidemic onset and mid-time were, on average, 30.4, 15.1, and 11.3 days earlier in 2007–2017 than in 1987–1996. The data were divided into three periods of 10, 10, and 11 years, and a discriminant analysis was performed to classify each year in the correct period. Using a sufficient subset of five discriminating variables, i.e., temperature and rainfall at harvest the previous fall, winter coldness, solar radiation, and disease incidence the previous year, it was possible to classify 93.5% of the ODM epidemics in the period where they belong, suggesting that the changes in *P. destructor* epidemiology may be related to variables associated with overwinter.

The second sub-objective was to develop a molecular-based assay specific to *P. destructor* allowing its quantification in environmental samples. In this study, a reliable and sensitive hydrolysis probe-based assay multiplexed with an internal control was developed on the internal transcribed spacer (ITS) region to quantify soil- and airborne inoculum of *P. destructor*. The assay specificity was tested against 17 isolates of *P. destructor* obtained from different locations worldwide, other Peronosporales, and various onion pathogens. Validation with artificially inoculated soil and air samples suggested an assay sensitivity of 1 sporangium m^{-3} of air and less

than 10 sporangia g^{-1} of dry soil. Validation with environmental air samples shows a linear relationship between microscopic and real-time qPCR counts. In naturally infested soils, inoculum ranged from 0 to 162 sporangia equivalent g^{-1} of dry soil, further supporting the hypothesis of overwintering under Northern climates.

The third sub-objective was to investigate the population structure of *P. destructor* at the landscape scale, using genotyping by sequencing. The study focused on a particular region of southwestern Quebec -Les Jardins de Napierville- to determine if the populations were clonal and regionally differentiated. The data was characterized by a high level of linkage disequilibrium, characteristic of clonal organisms. The null hypothesis of random mating was rejected when tested on predefined or non-predefined populations, indicating that linkage disequilibrium was not a function of population structure and suggesting a mixed reproduction mode. Moreover, these results reveal for the first-time patterns of heterozygosity indicative of an heterothallic reproduction system. Discriminant analysis of principal components allowed grouping *P. destructor* isolates by geographical regions, while AMOVA confirmed that this genetic differentiation was significant at the regional level. These results suggested that diversity in southern Quebec is essentially due to local mutations leading to a slow divergence of populations in time and space.

Together, these findings confirmed that the impact of climate change in southwestern Quebec, with warmer temperatures in the fall and milder winters, favored the survival of overwintering inoculum and thus modifying the ecology of *P. destructor* and therefore the epidemiology of onion downy mildew.

Résumé

Le mildiou de l'oignon, causé par *Peronospora destructor*, est une menace sérieuse pour les producteurs d'oignons du monde entier. Dans le sud-ouest du Québec (Canada) une augmentation régulière de l'occurrence des épidémies de mildiou est observée depuis le milieu des années 2000, atteignant 33% des champs d'oignons touchés en 2014. Sur l'oignon, *P. destructor* peut développer des infections locales et systémiques produisant de nombreux sporanges qui agissent comme inoculum initial localement, et pour les zones voisines. Plusieurs espèces de *Peronospora* sont transportées par le vent sur de courtes et longues distances, des climats chauds où elles survivent sur des plantes vivantes vers des climats plus frais. Dans l'est du Canada, on pensait que ce flux annuel de sporanges était la principale source de *P. destructor*. Cependant, il produit également des oospores capables de survivre à long terme. Dans cette thèse, l'hypothèse est que l'impact des changements climatiques dans le sud-ouest du Québec, avec des températures plus chaudes à l'automne et des hivers plus doux, favorise la survie de l'inoculum hivernant.

Le premier sous-objectif visait à étudier les variations dans l'incidence et l'apparition des épidémies de *P. destructor* et à identifier les variables météorologiques qui influencent son développement polyétique (c'est-à-dire sur plusieurs saisons) et ce en utilisant des données couvrant une période de 31 années. Les résultats de cette analyse ont montré que la date de la première observation et les temps pour atteindre 10 % et 50% de l'épidémie étaient, en moyenne, 30.4, 15.1 et 11.3 jours plus tôt en 2007-2017 qu'en 1987-1996. Les données ont été divisées en trois périodes de 10, 10 et 11 ans, et une analyse discriminante a été réalisée pour classer chaque année dans la bonne période. En utilisant un sous-ensemble suffisant de cinq variables discriminantes, c'est-à-dire la température et la pluviométrie à la récolte l'automne précédent, la rigueur de l'hiver, le rayonnement solaire et l'incidence de la maladie l'année précédente, il a été possible de classer 93.5 % des épidémies d'ODM dans la période à laquelle elles appartiennent, ce qui suggère que les changements dans l'épidémiologie de *P. destructor* peuvent être liés à des variables associées à l'hivernage.

Par la suite, un test moléculaire spécifique et sensible basé sur une sonde TaqMan multiplexée avec un contrôle interne a été développé sur la région ITS (Internal Transcribed Spacer) pour quantifier l'inoculum de *P. destructor* dans le sol et dans l'air. La spécificité du test a été testée contre 17 isolats de *P. destructor* obtenus dans différents endroits du monde, d'autres Peronosporales et divers pathogènes de l'oignon. La validation avec des échantillons d'air et de sol inoculés artificiellement a suggéré une sensibilité de de 1 sporange m^{-3} d'air et de moins de 10 sporanges g^{-1} de sol sec. La validation avec des échantillons d'air environnemental montre une relation linéaire entre les comptages microscopiques et la qPCR en temps réel. Dans les sols naturellement infestés, l'inoculum variait de 0 à 162 équivalents sporanges g^{-1} de sol sec, ce qui renforce l'hypothèse de la survie à l'hiver.

Le troisième sous-objectif visait l'étude de la structure des populations de *P. destructor* du sud-ouest de Montréal, en utilisant le génotypage par séquençage. Les données étaient caractérisées par un niveau élevé de déséquilibre de liaison, typique des organismes clonaux. L'hypothèse nulle de reproduction aléatoire a été rejetée lorsqu'elle a été testée sur des populations prédéfinies ou non, indiquant que le déséquilibre de liaison n'était pas une fonction de la structure de la population et suggérant un mode de reproduction mixte. De plus, ces résultats révèlent pour la première fois des patrons d'hétérozygotie suggérant un mode de reproduction hétérothallique. L'analyse discriminante des composantes principales a permis de regrouper les isolats de *P. destructor* par régions géographiques, tandis que l'analyse de variance moléculaire a confirmé que cette différenciation génétique était significative au niveau régional. Ces résultats suggèrent que la diversité dans le sud du Québec est essentiellement due à des mutations locales conduisant à une lente divergence des populations dans le temps et l'espace.

Ensemble, ces résultats démontrent que l'impact du changement climatique dans le sud-ouest du Québec, avec des températures plus chaudes à l'automne et des hivers plus doux, a favorisé la survie de l'inoculum hivernant, modifiant ainsi l'écologie de *P. destructor* et donc l'épidémiologie du mildiou de l'oignon.

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The journey to writing a thesis is laborious, long, and lonely. Conversely, it is also rewarding, challenging and somewhat addictive. In any case, this project would not have been possible without the support of many. My first thanks go to my co-supervisors, Dr. Odile Carisse, and Dr. Jean-Benoit Charron. Odile, thank you for your support, commitment, trust and for all the lengthy discussions we had throughout this journey. Jean-Benoit, thank you for your support, answering all my questions, and your opinion on various subjects (not always related to the project as such). I would also like to thank Guillaume J. Bilodeau for his wise advice, especially during the development of the qPCR assay, and for what will come. Finally, I sincerely thank Dr. Pierre Dutilleul for taking the time to explain many things to me. I once read that one could measure how much a professor cares when correcting the texts: the redder it returns, the more he cares. No matter if this adage is correct, I know you care. A special thanks to the members of the Charron Lab, especially Boris Mayer, who was there at the beginning with me. I would also like to acknowledge the funding and support I received from McGill, MAPAQ, Les Producteurs d'Oignons du Québec, and Phytodata. Finally, I would like to express my gratitude and love to my family, especially to you, Vero, and my daughters, Laurence, Camille, and Mathilde.

Preface and authors contribution

The following manuscript-based thesis was prepared in accordance with McGill University guidelines as provided by Graduate and Postdoctoral Studies. This thesis consists of seven chapters, with Chapter 1 as the general introduction; Chapter 3 is a literature review on aerobiology, which complements the primary literature review (Chapter 2), while Chapters 4-6 represent three research manuscripts. Chapter 3 is now published in Agronomy for Sustainable Development; Chapter 4 published in the special issue of Agronomy -Plant Disease Epidemiology: Changing Perspectives, Emerging Technologies, and Prediction Modeling; Chapter 5 published in Plant Disease, and Chapter 6 published in Molecular Plant Pathology. For all the chapters presented in this thesis, Hervé Van der Heyden (the candidate) benefited from significant contributions from its committee members: Odile Carisse, Jean-Benoit Charron, Pierre Dutilleul, and Guillaume J. Bilodeau.

HVH wrote the introduction, the literature review, and the general discussion. In Chapter 3, HVH and OC designed and planned the synthesis document. HVH and OC carried out the literature search and preparation of the draft manuscript. Critical review and comprehensive editing were performed by PD. J-BC and GJB revised the final version of the manuscript before submission.

In Chapter 4, HVH designed the study; HVH and PD designed the methodology; HVH performed the analysis with PD's guidance; HVH wrote the initial version; and PD, OC, J-BC, and GB revised the manuscript before submission.

In Chapter 5, HVH designed the study with input and advice from the applicant's committee (OC, J-BC, GB, and PD); HVH designed the methodology with valuable support from GB, performed the analysis, and wrote the initial draft. OC, J-BC, and GB provided critical input to the drafts and gave final approval for publication.

In Chapter 6, HVH designed the study with input and advice from its committee members (OC, J-BC, GB, and PD); HVH designed the methodology and performed the analysis with significant assistance from Dr. Marc-Olivier Duceppe in the use of bioinformatics pipelines and from PD in the statistical analyses. HVH wrote the initial version of the article, while P-OD, OC, PD, GB, and J-BC provided critical input to drafts and gave final approval for publication.

Contribution to original knowledge

Chapter 4 – Like all species, plant pathogens require specific environmental conditions to colonize and survive. These environmental conditions define their geographic distribution and the times of the year when disease outbreaks occur. With climate change, these conditions are likely to shift in time and space, allowing survival in places where it was not previously possible. Relying on data collected over 31 consecutive years, this research confirmed that onion downy mildew epidemics were significantly earlier in recent years and mostly influenced by precipitation regime, disease incidence of the previous year, warmer fall temperature during the harvest period the previous year, and warmer winters. In addition, this study is one of the few studies that aimed to characterize epidemics over a long period using observational data. These results provided evidence that *P. destructor* can overwinter in northern latitudes

Chapter 5 – The availability of accurate and sensitive measurement tools is essential for epidemiological and biosurveillance research. Chapter five describes the development of a reliable and sensitive assay based on a multiplexed hydrolysis probe with an internal control designed on the ITS (Internal Transcribed Spacer) region to quantify *P. destructor* inoculum in soil and air. Using this new molecular marker, this research provides among the first quantitative evidence of soil inoculum in southwestern Québec. In addition, the presence of *P. destructor* DNA in soils sampled in the spring suggests the presence of resting structures in these soils and supports the hypothesis of overwintering in northern climates.

Chapter 6 – As dispersal patterns and reproductive systems of organisms greatly influence the genetic diversity of their populations, evolutionary processes and demography can be inferred from population structure, which is defined by patterns of genetic diversity within and between populations. Thus, in Chapter 6, genotyping by sequencing was used to describe the genetic structure of *P. destructor* populations in southern Quebec. Genetic structure characteristic of clonal organisms, a significant effect of the region on genetic variation, and patterns of isolation by distances were identified. In other words, these results suggested that diversity in southern Quebec is essentially due to local mutations leading to a slow divergence of populations in time and space. Moreover, these results reveal for the first time patterns of heterozygosity which indicates heterothallic reproduction.

1 General introduction

1.1 Building on population biology to study overwintering of obligate biotrophic plant pathogens.

Throughout their life cycle, plants will be exposed to changes in the micro-environment, from normal variations in temperature or humidity to extreme temperature, excess precipitation, and drought. These changes also include the presence and abundance of microorganisms, whether beneficial or pathogenic (bacteria, viruses, fungi, or oomycetes). Together, these changes can significantly impact plant health, notably by influencing the occurrence and development of plant diseases. Although some definitions include diseases caused by abiotic factors (air pollutants, nutrient imbalance, mechanical impact, etc.) (Agrios 2005; Baudoin 2007), this body of work employs a narrower definition, only keeping diseases caused by microorganisms. Thus, in a strict sense, plant disease can be defined as any deterioration of the integrity of the plant caused by a microorganism that infects the plant and disturbs its normal development (Trigiano and Ownley 2016).

The onset of plant disease results from complex interactions between the plant, the pathogenic microorganism, and the environment (Zadoks and Schein 1979). This relationship, known as the disease triangle, has become the central theorem of plant pathology. Although the idea had been raised earlier, the concept of the disease triangle was formally introduced by McNew (1960) as a new method to study the relationships between the different factors that influence epidemics and provided, at the time, a new tool to understand how to predict them (Scholthof 2007). In his version of the disease triangle, McNew proposed six interacting factors: favorable environment, duration of the infection period, prevalence and virulence of the pathogen, age, and susceptibility of the host plant (McNew 1960). Today, modified versions of the disease triangle have also been proposed to include, for example, anthropogenic and time components (Agrios 2005; Scholthof 2007).

This relationship between the plant, the pathogen, and the environment has mainly been studied to describe and understand disease cycles during a growing season, the spatial and temporal distribution of pathogens, and to develop decision support systems (e.g. González-Fernández et al. 2020; Carisse et al. 2017; Carisse et al. 2012; Scherm and van Bruggen 1993; Hildebrand and

Sutton 1984c, a). For polycyclic diseases, epidemics are characterized by multiple generations of asexual spores, each cycle comprising several processes (i.e., germination, penetration, infection, tissue colonization, symptom development, sporulation, and dissemination) (Agrios 2005). Seasonal epidemics are generally characterized by a sigmoidal or "S-shaped" progression curve that ends with a plateau or decline in pathogen populations (Van der Plank 1963). For many pathogens, especially when within-host or saprophytic, survival is not possible beyond the growing season (e.g., for annual plants); this plateau or decline phase is consistent with the production of long-term survival structures, which are needed for the pathogen overwintering (Zadoks and Schein 1979).

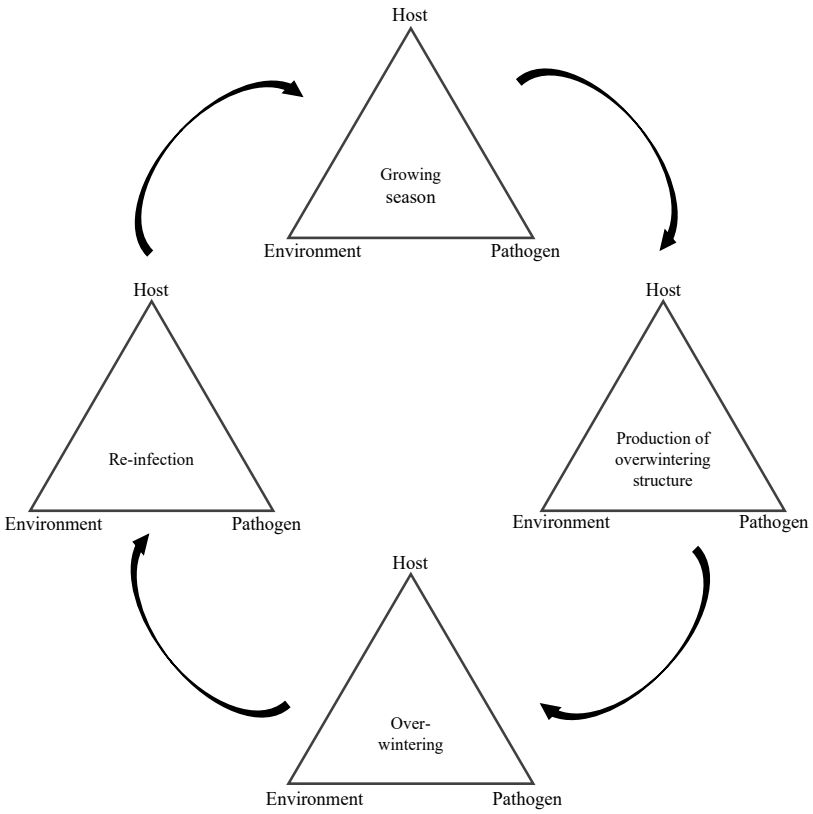

Figure 1-1. Disease triangle extended to the overwintering processes of the disease cycle. These processes include the production of overwintering structures, overwintering and reinfection. This framework emphasizes that all these processes are influenced by the environment, the host plant, and the pathogen. (Adapted from (Tack and Laine 2014).

In addition to the processes that occur during the growing season, disease cycles also include overwintering-related phases: production of survival structures, overwintering, and reinfection in subsequent seasons. As with processes that occur during the growing season, overwintering phases are also affected by interactions between the environment, the pathogen, and the plant. Thus, the disease triangle can be expanded to cover the polyetic aspect of epidemics, that is, the processes spanning several consecutive cropping seasons (Figure 1.1) (Tack and Laine 2014). However, the study of overwintering is complex, and knowledge about the production, maturation, and survival of overwintering structures is often limited. This is exacerbated for obligate biotrophic pathogens, such as *Peronospora* species responsible for several downy mildew diseases, as it is difficult to culture them and reproduce overwintering conditions. From an ecological and evolutionary point of view, these dynamic processes are of utmost interest and cannot be ignored (Tack and Laine 2014). Moreover, it is essential to understand how environmental factors influence the ability of pathogenic microorganisms to persist beyond a growing season, as this plays a critical role in determining the importance of disease outbreaks in subsequent seasons while shaping the genetic structure of local populations. Thus, to study overwintering related phases of these pathogens' life cycle, it is crucial to draw on various complementary disciplines borrowed from epidemiology and population genetics (Milgroom 2017; Milgroom and Peever 2003).

Among the strategies advocated for studying overwintering-related phases in obligate biotrophs, one can consider analyzing observational data collected during several consecutive years, such as scouting data. This way, the polyetic nature of plant disease epidemics is taken into account and contributes to inferring the key factors that drive pathogen carryover from one season to the next (Zwankhuizen and Zadoks 2002). However, the use of this type of data presents a real challenge when it comes to analysis. This is due to the data themself since they are generally not collected for these purposes (test of hypothesis), introducing additional sources of bias while representing the field reality (Garrett 2013). Moreover, using this type of data requires accepting correlations without necessarily understanding the underlying causes (Garrett 2013). Nevertheless, the results of long-term observational studies put short-term studies into perspective and, more importantly, provide guidelines for future work (Zwankhuizen and Zadoks 2002).

Being able to detect pathogen inoculum in different types of environmental samples taken from various locations (space) and at different times allows, among other things, to infer dispersal, survival, and overwintering patterns. For this, molecular approaches have proven their usefulness

in dealing with complex research questions. These molecular tools, especially real-time qPCR, allow detection in a wide variety of sample types, whether in air, water, soil, or plant tissue. Real-time qPCR has been used, for example, to study the role of oospores in over-wintering and seed transmission of *Peronospora effusa* and *P. arborescens* (Kunjeti et al. 2015; Montes-Borrego et al. 2010; Montes-Borrego et al. 2009) or to estimate the number of oospores in grapevine leaf litter (Si Ammour et al. 2020), in addition to monitoring of populations (Bello Rodriguez et al. 2020; Rahman et al. 2020; Summers et al. 2015a; Gent et al. 2009).

Dispersal, sources of inoculum, and mode of reproduction of organisms greatly influence the genetic diversity of their populations. Hence, evolutionary processes and demography can be inferred from population structure, which is defined by patterns of genetic diversity within and between populations (Milgroom 2017). Five evolutionary forces contribute to shaping populations: mutation, genetic drift, reproduction and mating, gene flow, and selection (McDonald 2004). According to the Hardy-Weinberg law, when reproduction is random in a population, and evolutionary forces do not influence the population, the population is in equilibrium (McDonald 2004; Milgroom 2017). In other words, when the population is in equilibrium, the allelic and genotypic frequencies are constant from one generation to the next (McDonald 2004; Milgroom 2017). Thus, population genetic studies attempt to determine if a population is in equilibrium and, if not, to find out why. Population genetics has been applied, for example, to understand the migration patterns of *Peronospora tabacina* in Europe and North America (Blanco-Meneses et al. 2018), to identify host-specific clades and implement clade-specific monitoring of *Pseudoperonospora cubensis* inoculum (Wallace et al. 2020; Rahman et al. 2020; Summers et al. 2015b; Quesada-Ocampo et al. 2012) or to confirm overwintering of *P. humuli* in hop fields (Gent et al. 2019).

Peronospora destructor, responsible for onion downy mildew (ODM), is one of those obligate biotrophic pathogens for which information on epidemiology and population genetics is limited, particularly regarding overwintering survival. Moreover, the few studies explicitly concerning this aspect of its epidemiology date back to the 1950s (Cook 1932; McKay 1957). Thus, to fully understand the impact of overwintering on pathogen ecology and population structure, we must shift our focus from a one-season to a polyetic conception of epidemics that includes seasonal dynamics and the production and survival of overwintering structures.

1.2 *Objectives and hypotheses*

Peronospora destructor is generally considered a periodically introduced pathogen (i.e., a pathogen that appeared sporadically and disappeared for several years between outbreaks (LaMondia and Aylor 2001)). However, over the last decade, onion downy mildew outbreaks have become more frequent in southwestern Quebec, and epidemic severity increases. Thus, the overarching objective of this research was to investigate the overwintering of *P. destructor* in southwestern Quebec. Through a population biology approach, combining epidemiology and population genetics, the question is to determine whether *P. destructor* survives the winter in southwestern Quebec and, if so, what are the variables that most influence these changes and what is the impact on the population structure.

1.2.1 Chapter 4

The main objective of chapter 4 is to investigate, using observational data collected over 31 consecutive years, the variations in onion downy mildew incidence and epidemic onset and identify the meteorological variables that influence its polyetic development.

Hypothesis

Changes in climatic conditions favor the overwintering of *P. destructor* and, therefore, the frequency, duration, and precocity of ODM epidemics.

Specific objectives

- Characterize the seasonal distribution patterns of onion downy mildew epidemics.
- Determine if the onset of seasonal epidemics comes earlier and disease incidence is more critical.
- Identify the variables influencing the polyetic nature of onion downy mildew development.

1.2.2 Chapter 5

In chapter 5, the main objective was to develop a specific multiplex real-time hydrolysis probe qPCR assay for *P. destructor* identification and quantification in soil or air samples.

Hypothesis

Because internal transcribed spacer (ITS) is used as genetic barcodes for oomycetes, it could be used to develop a molecular marker to detect and quantify *Peronospora destructor* from air and soil samples.

Specific objectives

- To develop a qPCR assay specific to *P. destructor*.
- To combine the primary qPCR assay with an internal control system to detect PCR inhibition.
- Through the validation process, assess the presence of *P. destructor* inoculum in air and soil.

1.2.3 Chapter 6

In chapter 6, genotyping by sequencing was used to investigate the population structure of *P. destructor* at the landscape scale.

Hypothesis

Although *P. destructors* populations are expected to be predominantly clonal, these will be differentiated at a regional scale if *P. destructor* survives the winter locally.

Specific objectives

- To estimate the levels of genetic diversity and differentiation between and within populations.
- To determine if the populations were clonal and regionally differentiated.
- To analyze the relationship between genetic and geographic distance.

2 Literature review

2.1 Onion production

Nowadays, onion is the third most cultivated vegetable crop worldwide, tied with tomato (FAO 2017). Over the last 20 years, world onion production more than doubled, moving from 2.4 million hectares in 1997 to 5.3 million hectares of land (FAO 2017). This commodity was cultivated in 154 countries in 2014, generating revenues of about $4 billion (FAO 2017). Among these countries, China, India, and the USA produce more than 50% of the world's onion production (FAO 2017). In Canada, Ontario and Québec grow more than 80% of the production, with 2299 and 2309 ha dedicated to this crop, respectively (Statistics Canada, CANSIM 001-0013, accessed November 8, 2021). The main types of onions grown in Canada are white, red, and yellow globe onions, but in Québec, most onions grown are yellow globe cultivars. Most of the onions grown in Québec are sown, while a small share of the production is grown from transplants or dry sets. Even if onions can be produced in various types of soils (Brewster 2008), most of the onions are produced in histosols or muck soils. In southwestern Quebec, seeding or transplantation is generally performed in late April to mid-May when the ground is thawed. Onions are sown about 2-2.5 cm below the soil surface, on 1.8 m wide beds, with double rows spaced 45cm apart.

2.2 The disease: onion downy mildew

Like many crops, onions are threatened by pests and pathogens belonging to various taxa. On the insect side, the most important pests are probably onion thrips (*Thrips tabaci*) (Leach et al. 2020) and onion and seed corn maggots (*Delia antiqua*, *D. platura*, and *D. florilega*) (Moretti et al. 2021). Bacterial rots are also of great economic importance; several species being responsible for bacterial rots, including *Burkholderia* spp. and *Pantoea* spp. (Zaid et al. 2012). Viruses are also responsible for important diseases like Iris yellow spot virus (Gent et al. 2006; Hoepting et al. 2008). Fungi represent the group with the largest number of pathogenic species. The most important fungal pathogens of onion are *Botrytis squamosa*, responsible for botrytis leaf blight; *Botrytis cinerea*, responsible for leaky spot and crown rot; *Fusarium oxysporum* f. sp. *cepae*, responsible for Fusarium basal rot; *Stemphylium vesicarium*, responsible Stemphylium leaf blight; *Alternaria porri*, responsible for purple blotch; *Cladosporium allii-cepae*, responsible for leaf blotch, and *Botrytis spp.* responsible for neck rot (Maude 1998). Although most onion pathogens

are found in fungi, the most devastating is unquestionably onion downy mildew caused by the oomycete *Peronospora destructor* (Palti 1989).

2.2.1 Onion downy mildew symptoms and distribution

Onion downy mildew (ODM) is a widespread disease that is often difficult to manage and can be destructive, with yield losses reaching 75% (Develash and Sugha 1997; Gerald and Obrien 1994; O Brien 1992). It has been reported on all continents, especially those with temperate climates (Palti 1989). Symptoms appear most often on healthy leaves, with infected areas turning pale, yellow, and eventually collapses. The lesions are usually covered with abundant sporulation, giving the leaves a purple color (Figure 2.1 A-C). Signs of ODM can also be seen on older leaves when they are wet or when humidity is high: fine hairy excrescences of grayish color are then perceived on the surface of the leaves (Palti 1989). In addition to affecting foliage, onion downy mildew can also affect flower stems and bulbs systemically (Palti 1989). Infected bulbs will soften and may rot in storage. In addition, ODM lesions are often affected by secondary pathogens such as *S. vesicarium* or *A. porri*, which contribute to plant dieback and make the diagnosis more complex. At the field level, mildew symptoms appear as small foci of yellowed plants that enlarge

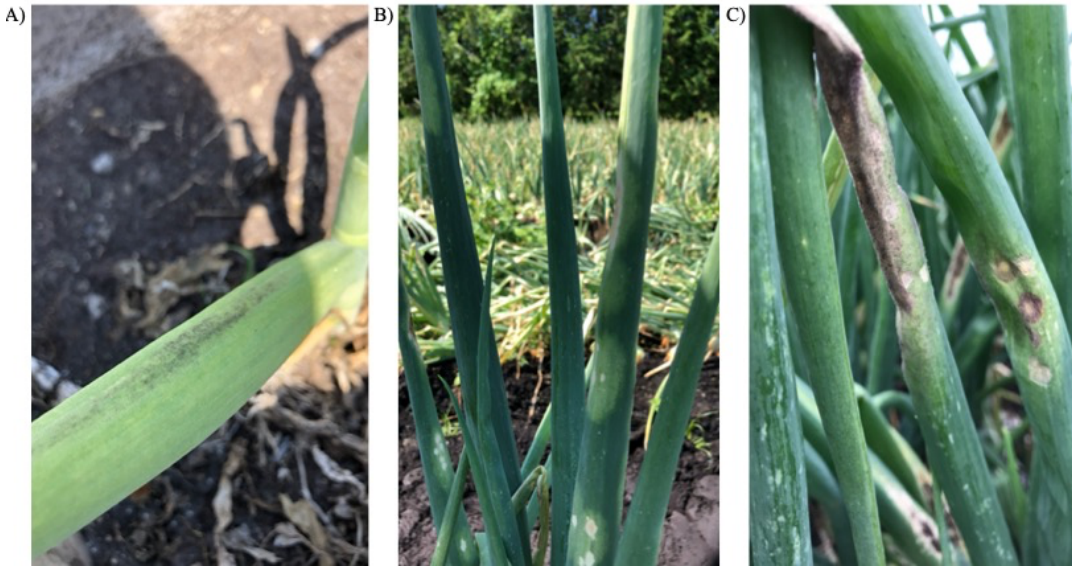

Figure 2-1. Common symptoms of *P. destructor* on onion plants. In A) sporulation observed on volunteer plants in the spring, B) oblong lesions showing marked discoloration of leaf tissue and C) more advanced lesions showing abundant sporulation and heavy dieback (Pictures taken by H. Van der Heyden).

with each disease cycle, generally in the direction of the prevailing winds. *Peronospora destructor* infects only species of the Allium genus, mainly onions, shallots, and bunching onions.

2.3 A susceptible host

2.3.1 The genus Allium L.

The genus Allium L. includes about 800 species, making it one of the most important genera among the monocotyledons (Friesen et al. 2006). *Allium* species are widespread throughout the northern hemisphere, from the dry subtropics to the boreal regions (Friesen et al. 2006). The genus consists mainly of perennial plants characterized by an umbel inflorescence, storage bulbs composed of membranous to fibrous tunics, and the production of a large amount of cysteine sulfoxides responsible for the typical smell and taste (Li et al. 2010). Among *Alliums*, the primary chromosome number varies between 7 and 11 (average of 8), while the levels of ploidy are highly diversified (from diploid to octoploid species) (Baranyi and Greilhuber 1999; Han et al. 2020). *Allium* species have been grouped in different subgenera according to their visible characteristics and, more recently, based on plastid DNA and internal transcribed spacer region (ITS) sequences (Friesen et al. 2006). Section *Cepa* is probably the most important one from an economic point of view. It includes commonly cultivated species such as Japanese bunching onion (*A. fistulosum*) and dry bulb onion (*A. cepa*) (Gurushidze et al. 2007).

2.3.2 Allium sect. cepa

Allium section Cepa is a relatively small group comprising ten wild species in addition to the cultivated species (*A. fistulosum* and *A. cepa*). The wild species grow naturally in Asia, between Siberia, the Persian Gulf, and the Caspian Sea. It is generally accepted that onions have been cultivated for thousands of years and do not exist as wild species. It is believed that Sumerian civilizations first domesticated onion in northern Iran and Turkmenistan (Brewster 1994). However, since onion tissue leaves little or no trace, it is difficult to determine its precise origin. Southwestern Asia is considered to be the primary center of domestication and variability. In contrast, other regions of remarkable diversity like the Mediterranean and North America are considered secondary centers of origin (Gurushidze et al. 2007; Nguyen et al. 2008).

For several years, hypotheses have been advanced concerning the phylogeny of this group and concerning its domestication. The species *A. oschaninii*, native to Central Asia, was first proposed

as an ancestor of the cultivated onion. Still, the two species were found to have different chromosomal banding patterns and significant interbreeding barriers *(Raamsdonk et al. 1992)*. However, in the same study, Raamsdonk et al. (1992) report having successfully crossed *A. cepa* with *A. vavilovii*, a species found in the Kopet-Dagh mountains of Turkmenistan with phenotypic similarity to *A. oschaninii*. Moreover, sequencing of the ITS region identified three genetic groups in the section *cepa* (1- *A. altaicum* and *A. fistulosum, 2- A. farctum, A. roylei, A. asarense, A. cepa,* and *A. vavilovii,* and *3- A. galanthum, A. oschaninii, A. praemixtum* and *A. pskemense*), supporting the hypothesis that the closest wild relative of *A. cepa* was *A. vavilovii* (Gurushidze et al. 2007). Thus, this species has long been considered the most likely ancestor of the cultivated onion. However, the results of a recent study, based on the complete sequencing of the chloroplast genome of species in section *Cepa*, suggests two clades instead and would group *A. cepa* with *A. galanthum* and *A. altaicum (Yusupov et al. 2020)*. Furthermore, this study suggests that the wild species from Central Asia would not have played any role in the domestication of cultivated onion, contradicting the previous hypothesis. Thus, despite these advances, phylogenetic uncertainties remain in section *Cepa*.

2.3.3 Host susceptibility to Peronospora destructor

The quest to discover resistance to *P. destructor* in *Allium cepa* has been ongoing for several decades (Kofoet et al. 1990; Kofoet and Zinkernagel 1990). This research made a significant step forward when van der Meer and de Vries (1990) reported that after severe epidemics of downy mildew, no symptoms were observed in *A. roylei* Stearn, a species closely related to *A. cepa*. *Allium roylei* was successfully crossed with *A. cepa*, resulting in a partially fertile interspecific hybrid (van der Meer and de Vries 1990). This discovery finally suggested the possibility of using the genetics of *A. roylei* in onion breeding programs. Since the hybrids obtained from these crosses were completely resistant and the backcrosses of these hybrids were segregated in a 1:1 ratio, the authors suggested that resistance to onion downy mildew was conferred in *A. roylei* by a single dominant locus, named Pd1 (Kofoet et al. 1990). This finding was later supported by random amplified polymorphic DNA markers (RAPD) and by using species-specific sequence-characterized amplified region markers (SCAR) (de Vries et al. 1992; van Heusden et al. 2000b; van Heusden et al. 2000a)

Since the identification of Pd1, obtaining homozygous introgression lines has proven to be difficult (Scholten et al. 2007). Over the years, markers for resistance in *P. destructor* have become less efficient and have lost their discriminatory power over time. However, the introgression of the onion downy mildew resistance gene has been reported in cultivated onions (Scholten et al. 2007). The seed company Hazera holds a patent on this resistance (EP1819217A1), and one downy mildew resistant onion variety has recently entered the Canadian market (Hackero cultivars).

2.4 A virulent pathogen

2.4.1 Oomycetes

Peronospora destructor Caspary Berk is an obligate biotrophic organism belonging to the oomycetes; biflagellate organisms known as stramenopiles, placed within the Chromalveolates supergroup (Keeling 2009). It has been suggested that oomycetes diverged from diatoms about 500 million years ago (Matari and Blair 2014). Oomycetes have evolved in parallel with true fungi and, like the latter, grow by forming tip-branching mycelium and have developed similar ecological roles and nutrient patterns (Beakes et al. 2012). However, unlike true fungi, oomycetes contain cellulose and have glucans as a component of their cell walls instead of chitin compounds (Thines 2014). They also differ from fungi in their lysine biosynthesis pathway and have two flagella in their zoospores, contained in sporangia (Beakes et al. 2012). The formation of oospores in oomycetes is also a unique characteristic compared to fungi (Thines and Choi 2015). Many species have yet to be described, and the phylogeny of the oomycetes is evolving rapidly; however, they can be split into the basal lineage and the crown groups (McCarthy and Fitzpatrick 2017). To date, most known species belong to the crown groups, more precisely within the *Saprolegniomycetes* and *Peronosporomycetes* branches (Thines 2014). With the exception of a few species, saprolegnians are parasites of animals or saprophytes, whereas the majority of phytopathogenic oomycetes are found in the peronosporaleans lineages more precisely in the order Albuginales and Peronosporales (Thines 2014). The Peronosporales include important organisms from an economic point of view, such as *Pythium* spp., *Phytophthora* spp., and of course, those responsible for downy mildews (Thines 2014; Thines and Choi 2015; Beakes et al. 2012).

2.4.2 The downy mildew pathogens

With more than 700 species belonging to 19 genera, downy mildews represent almost one-third of all oomycetes. They can be grouped into three major monophyletic groups: species with

pyriform haustoria, brassicolous downy mildews, and downy mildew with colored conidia (Thines and Choi 2015). The downy mildews with piriform haustoria contain eight genera, six genera restricted to Asteraceae (*Basidiophora*, *Benuta*, *Bremia*, *Novotelnova*, *Paraperonospora,* and *Protobremia*), the genus *Plasmoverna* restricted to *Ranunculaceae,* and the genus *Plasmopara,* which has a wide range of eudicot hosts (Thines and Choi 2015). The brassicolous downy mildews contain only two genera, *Hyaloperonospora* and *Perofascia,* and are almost restricted to the Brassicaceae. The group of downy mildews with colored conidia, formed by the genera *Peronospora* and *Pseudoperonospora*, contains the largest number of species, while the genus *Peronospora* is the most important of all Peronosporaceae. They are found in many eudicots in addition to some monocots like Alliaceae and are considered a monophyletic genus (Thines and Choi 2015).

Species of the genus *Peronospora* are highly host-specific, and it has been suggested that their evolutionary success may be linked to the evolution of melanized sporangia, which would allow them to survive longer during long-distance transport when exposed to solar radiation (Lebeda and Schwinn 1994). Another important feature of *Peronospora* evolution is the frequent host jumps between non-related families (Voglmayr 2003; Choi and Thines 2015). Among the most recent

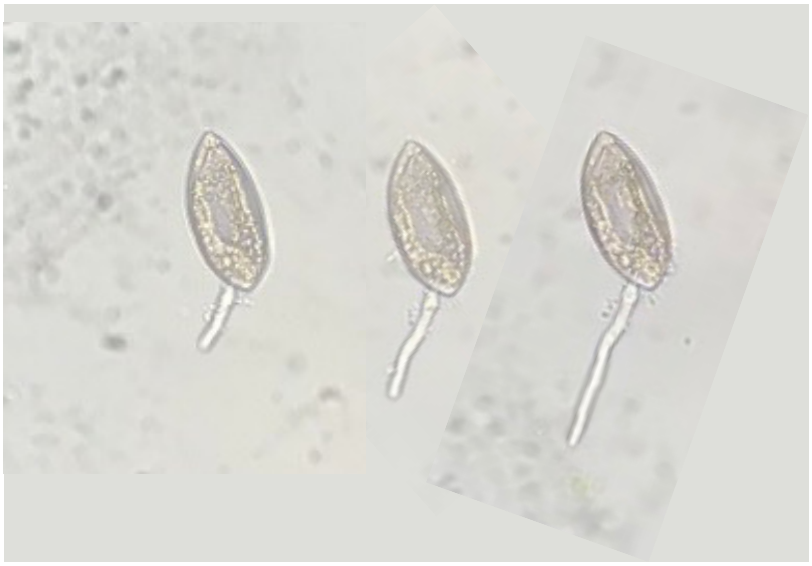

Figure 2-2: *Peronospora* destructor sporangia showing an elongating germ tube. The pictures were taken at a 30-minute interval (Pictures taken by H. Van der Heyden).

hosts, or at least the most noteworthy, is the jump from the dicots (probably *Chenopodiaceae*) to the *Alliaceae*, giving rise to *P. destructor* (Voglmayr 2003).

2.4.3 Peronospora destructor

Peronospora destructor was first described in England in 1841, first as *Botrytis destructor* Berk. (Berkeley 1841) than as *Peronospora destructor* (Berk.) Casp. ex Berk. (Berkeley 1860). It is now widespread throughout virtually all onion production areas of the world. Sporangiophores are nonseptate and harbor various shades of purple. They usually emerge from stomata and measure 122-150 µm long by 7-18 µm wide at their base. They taper to acute sterigmata at the tips, 2-6 times monopodially branched, bearing 3-63 sporangia (Yarwood 1943). Sporangia are pyriform to fusiform and are attached to the sporangiophore by a narrow-pointed tip. They measure 18-29 µm wide by 40-22 µm long, have thin walls, and are slightly papillate at the proximal end. Germination of sporangia occurs through 1 or 2 germ tubes (Figure 2-2). The mycelium is also nonseptate, 4-13 µm in length, and unfolds intercellularly (Yarwood 1943). Haustoria are filamentous, coiled within the cells, and measure 1.5-5 µm in diameter. Oogones measure between

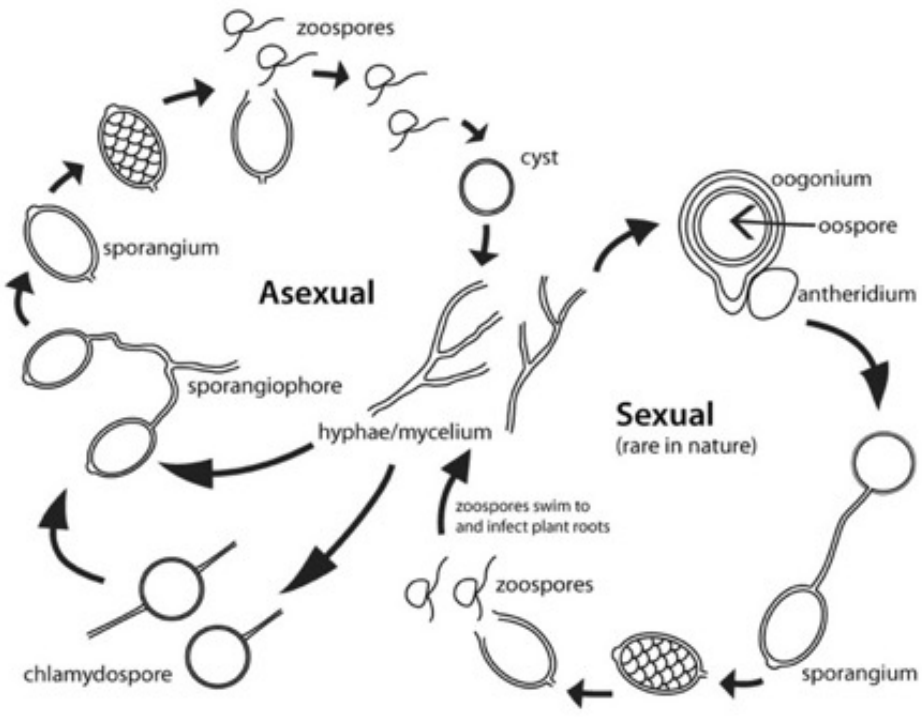

Figure 2-3: Typical life cycle of oomycetes, illustrating the asexual and sexual phases. (From Abad et al. 2019).

43 and 54 µm in diameter, while oospores, often numerous, are globular and measure between 30 and 44 µm in diameter (Yarwood 1943). Conditions that favor infection, sporulation, production of survival structures, and dispersal are covered in section 2.4.

2.4.4 Oomycete's life cycles

The life cycle of oomycetes generally includes two main cycles. The first one is asexual and results in sporangia production containing motile zoospores with two flagella. In contrast, the second cycle is sexual and leads to the production of oospores, their long-term resting structures (Figure 2-1).

2.4.4.1 Asexual reproduction

As with many true fungi, asexual reproduction in oomycetes is essential since the sporangia produced are their main mean of dispersal over short and long distances (Aylor et al. 1982). Sporangia production is usually induced when nutritional conditions limit mycelial growth and are limited by various environmental conditions (Hardham 2009). Sporangia are multinucleate structures, usually oval with or without papillae, and are produced from the mycelium directly at the tip of the growing hyphae or the top of sporangiophores. These sporangia can germinate directly or release motile zoospores, except for certain species like *Bremia lactucae* and *Hyaloperonospora arabidospis* which have lost the ability to produce zoospores (Hardham 2009). Some species can also form chlamydospores as asexual resting structures. Reproduction typically includes two phases, sporangiogenesis, during which the multinucleate sporangia are produced, and zoosporangenesis, during which uninucleate zoospores are assembled.

In sporangiogenesis, sporangia differentiate directly from the tip of the hyphae or following the formation of sporangiophores (Hardham 2009). The formation of this structure begins with the cessation of hyphal extension and the displacement of the cytosol towards the enlarging hyphal tip (Hardham 2009). In Saprolegniales, sporangia are cylindrical enlargements of the hyphal apex, while in Peronosporales, sporangia are spherical to football shaped. During sporangia formation, most nuclei are transferred from hyphal tips to the forming sporangia. These nuclei are generally organized regularly in the cytoplasm and held in place by microtubules originating from the base and spread throughout the cytoplasm. During sporangiogenesis, zoospore-specific motility-related components, vesicles, and membrane compartments are formed in the hyphae and migrate into the

forming sporangia (Hardham 2009). It is, however, during zoosporogenesis that the division of mature sporangia into uninucleate zoospores is organized.

Sporangia can germinate directly without the formation of zoospores, but certain conditions, notably cool temperatures, high humidity, and pH, trigger the differentiation of zoospores within the mature sporangia (Kong et al. 2009). Once differentiated, the zoospore can be released, and for most oomycetes, this occurs by rupture of the apical papilla of the sporangia (Hardham 2009). This rupture is caused by the water pressure that increases inside the sporangia, caused by the accumulation of a gelatinous substance between the zoospores and the sporangia wall (Gisi and Zentmyer 1980). With these two flagella, the zoospores can move on the surface of the host plant tissues. They are attracted to the candidate host by detecting chemical and electrical gradients, specific or not. When they have reached a suitable area, the zoospores become immobile and rapidly encyst, lose their flagella, become rounded and produce a cellulose cell wall, adhesive compounds, and other extracellular matrix elements (Hardham 2009). It is important to note that species of the genus *Peronospora* do not produce zoospore and that sporangia germinate only through germ-tubes (Thines and Choi 2015).

Germination of oomycete spores (encysted zoospores and sporangia) begins with the germination of a germ tube, usually on the side that faces the plant surface (Deacon and Donaldson 1993). The germ tube growth can be chemotropically oriented to a site of penetration (Hardham 2009). Next, germ tubes grow and develop through mitosis to form somatic hyphae. These hyphae can develop appressorium-like swellings capable of penetrating host tissue. Like the appressoria of fungi, appressoria-like structures of oomycetes are specialized cells capable of exerting pressure that facilitates penetration of the plant surface (Grenville-Briggs et al. 2008). In addition to using a physical method under the effect of appressoria, the hyphae of oomycetes also synthesize and secrete enzymes capable of degrading the components of the cell wall of the plant, namely, pectin, cellulose, and glucans (Boudjeko et al. 2006). In addition, they also secrete proteins that protect themselves from the plant's natural defenses, such as glucanase and protease inhibitors (Damasceno et al. 2008).

The progression of the mycelium after penetration of the tissues takes place through the growth of inter- and intra-cellular branched mycelium (Hardham 2009). During mycelial development, contact of the mycelium with parenchymal cells triggers specialized feeding structures, the

haustoria. As haustoria develop, the cell wall of the cell is locally degraded, and small hyphae penetrate the cell and invade the plasma membrane of the plant cell (Enkerli et al. 2011). In necrotrophic species, tissue colonization results in cell death to facilitate access to the nutrients it contains. For biotrophic species, the destruction of host cells is less rapid because these species need to establish a stable relationship with the host plant (Chambers et al. 1995). The colonization of the host by these necrotrophic or biotrophic oomycetes ends with the formation of new sporangia and the beginning of a new asexual cycle.

2.4.4.2 Sexual reproduction

For most oomycete species, asexual reproduction cycles are interspersed with sexual reproduction, characterized by the production of oospores. This diploid thick-walled cell can survive in adverse environmental conditions such as frost and drought (Judelson 2008). Sexual reproduction requires, for homothallic species, the presence of two sexual types (or mating types) while heterothallic species are capable of sexual reproduction within the same culture (Judelson 2008). Sexual reproduction begins with the formation of female and male gametangia (antheridia and oogonia) generally as a non-differentiated swelling (or thallus) from the hyphal tips (Fabritius et al. 2002). The oogonium develops typically without septation, while a septum delimits the antheridium (Judelson 2008). After the contact of the sexual organs, generally facilitated by adhesive substances secreted by the antheridium, synchronized meiosis is observed in each, followed by the penetration of a fertilizing tube between the antheridium and the oogonium (Judelson 2008). A haploid nucleus is transferred from the male to the female, which contains a dozen gametic nuclei. Only one of these nuclei will be fertilized. After fertilization, a plug separates the thallus and the cytoplasm of the oogonia, which is gradually enriched in lipids, proteins, and glucans and inside which a vacuole rich in phosphate, called the ooplast, is formed. The oospore then matures, resulting in a thickening of the cell wall and the disappearance of ribosomes and cytochromes (Judelson 2008).

Sexual reproduction is complex in oomycetes as it is influenced by several factors, including the mating system (homothallic or heterothallic), the production of male and female gametangia, and the environment. In heterothallic species, both individuals must be of a different mating type; some are bisexual (able to produce both types of gametangia) while others can only produce one or the other. On the contrary, homothallic species can produce both sexual types but can also mate

with another isolate and can even show some sexual preferences (Judelson 2008). Male and female gametangia production could also be regulated by certain hormones (antheridiol produced by females and oogoniols produced by males) (McMorris et al. 1978), but only a few studies have addressed this topic. The best-documented systems for mating types are probably those of *Phytophthora* spp. For these species, the A1 and A2 mating types are distinguished by producing and detecting certain hormones, while the detection of the opposite hormone establishes compatibility (Ko 1988).

The environmental conditions that favor or not the development of oospores is variable from one genus to another and is undoubtedly associated with the occupied ecological niche. For plant pathogenic species, a decrease in the carbon-nitrogen ratio could stimulate oosporogenesis (Judelson 2008). Some environmental factors, such as physical damage, fungicides residues, plant exudates, or other organisms, could also favor oospore production through selfing or secondary homothallism (Groves and Ristaino 2000). Generally, conditions that favor asexual reproduction disfavor oosporogenesis and vice versa (Fabritius et al. 2002). The environmental factors that favor the production of long-term survival structures are further reviewed in Section 2.2.3.

Oospores are robust structures that can survive for long periods in hostile environments. However, these structures must often go through a long constitutive dormancy, sometimes several years, before they can germinate. This is especially true of *P. destructor*, which is known to survive in soils for over 25 years (McKay 1957). This state of reversible dormancy could be caused by the action of auto-inhibitors or a barrier effect for the entry of nutrients (Sussman and Douthit 1973). Breaking this dormancy can be difficult, and while some species can germinate immediately, some require a few days and others several years. In addition, breaking dormancy can be so arduous that many oospores do not germinate readily even when conditions are favorable (Judelson 2008). However, this represents an adaptive advantage since the resting inoculum is not entirely depleted during a single favorable event, ensuring a certain persistence of resting populations (McKay 1957).

2.4.5 Effect of reproduction system on population structure

There are five evolutionary forces that influence patterns of genetic diversity within populations: i.e., migration, genetic drift, mutation, recombination, and selection (McDonald and Linde 2002). During migration, there is a movement of individuals between subpopulations, which

results in gene flow that contributes to the enrichment of the local gene pool (Goodwin 1997). Conversely, with random genetic drift, the result is a decrease in genetic diversity. It refers to the fluctuation in the number of genetic variants in populations, with infrequent alleles being more likely to disappear in small populations (Ellstrand and Elam 1993). Thus, when genetic drift begins, it will continue until a given allele disappears completely from a population or is the only one present at a given locus (Milgroom 2017). On its side, mutation is considered as a weak evolutionary force on its own but is the source of all genetic variations. It can be defined as any heritable change in the genome of an organism. In contrast, recombination is a much stronger evolutionary force, and it corresponds to exchanges of nucleotides between two genomes, either of whole chromosomes or smaller chromosomes segments (Grünwald et al. 2016). Under specific conditions, recombination can result in new multilocus genotypes (MLGs) corresponding to new combinations of alleles at different loci. Moreover, recombination purges deleterious mutations and produces progeny free of these. Finally, when these genetic and phenotypic variations influence fitness, selection pressures will modify the frequency of the underlying alleles (Milgroom 2017). This selection, natural or artificial, represents a powerful evolutionary force. However, these forces usually do not act alone. They act together, in synergy or as antagonists, and when they interact, one can expect that organisms will adapt to their changing environment (Milgroom 2017).

For most oomycetes, sexual reproduction plays an essential role from an ecological point of view. When it occurs, it results in the formation of oospores, allowing the organism to survive for an extended period. Otherwise, in the absence of sexual reproduction, the survival of oomycetes is only possible in the form of mycelium in living tissues (e.g., onion bulbs for *P. destructor*) (Hildebrand and Sutton 1980). In addition to producing a long-lasting source of inoculum and allowing obligate biotrophs to persist without their host for an extended period, sexual reproduction is the main source of genetic recombination. In organisms where sexual reproduction takes place, the structure and genetic diversity of populations are characteristic. In this case, each individual of a population is unique because of the exchange of genetic material during meiosis and will develop unique MLGs (Milgroom 2017). Hence, when enough polymorphic genetic markers are used, the probability of sampling the same genotype more than once is quite low (Grünwald et al. 2016). Recombination increases genotypic diversity but does not influence genetic or allelic diversity. In sexually reproducing populations, when there are no restrictions on

mating, whether associated with genetics, mating types, host specialization, isolation by distance, reproduction is considered random (Milgroom 2017). In a randomly mating population, if all individuals of the opposite mating type have the potential to reproduce, and if there is no selection pressure, migration, drift, or mutations, such a population is in equilibrium, according to Hardy-Weinberg theory (Grunwald et al. 2003). According to this theory, the genetic variation within a population remains constant from one generation to the next if no interfering factors occur. Thus, when reproduction is random, and there are no disturbing factors, Hardy-Weinberg's law predicts that allelic and genotypic frequency will remain constant because they are in equilibrium (Table 2-1).

Table 2-1. Genetic characteristics of sexually and asexually reproducing populations (Adapted from Milgroom (2017)).

	Random mating	**Asexual reproduction**
Recombination among loci	Expected frequencies of MLGs	MLGs overrepresented
	Presence of recombinant MLGs	Absence of recombinant MLGs
	Linkage equilibrium	Linkage disequilibrium
	No correlation among markers	Correlation among markers
Segregation of alleles	Hardy Weinberg equilibrium	Deviation from Hardy-Weinberg equilibrium
	Presence of all segregating genotypes	Absence of segregating genotypes
	Expected heterozygosity	Excess heterozygosity

Completely random reproduction is rare and is located at one end of the reproductive spectrum. Many polycyclic oomycetes often undergo multiple asexual cycles followed by occasional sexual cycles. Hence, at the other end of the reproductive spectrum is asexual reproduction. Strict asexual reproduction results in a clonal population structure because each individual is genetically identical to the parents, except for mutations (Milgroom 2017). These mutations can represent many heritable changes, from simple substitutions due to replication error (SNP) to chromosomes rearrangement. They are the source of all genetic variation, without which there can be no genetic variation on which evolutionary processes can operate (McDonald and Linde 2002). In theory, strict asexual reproduction is considered an evolutionary dead-end since deleterious mutations cannot be purged without recombination (Milgroom 2017). The accumulation of deleterious mutations gradually reduces fitness which in the end can lead to population extinction. In the short

term, however, asexual reproduction has certain advantages. Species with a high mutation rate can also adapt rapidly to changing environments or bypass host resistance (McDonald and Linde 2002). In addition, since each parent transmits 100% of its genome to its descendants (clones), favorable allelic combinations are transmitted more rapidly within populations. Hence, mutations that accumulate within a clonal population can generate closely related but genetically differentiate MLGs. This variation within a clonal population is known as clonal lineage, which includes all the descendants of the same MLG. In clonal populations, a lack of recombination leads to the overrepresentation of certain MLGs, linkage disequilibrium, and correlation between different independent markers (Table 2-1) (Milgroom 2017). Thus, in diploid organisms such as oomycetes, a lack of segregation triggers a deviation from the Hardy-Weinberg equilibrium or linkage disequilibrium, which can be interpreted along with other characteristics to infer information about reproductive biology (Grünwald et al. 2016).

Examples of inference about sexual reproduction from population genetics using either microsatellites or genotyping by sequencing are numerous for *Phytophthora* species. The most studied on the subject is probably *Ph. infestans*, responsible for potato late blight, for which the population structure is mainly clonal. Hence, seasonal outbreaks are usually caused by clonal lineages sometimes having different patterns of aggressiveness (Blandón-Díaz et al. 2011; Hansen et al. 2016; Knaus et al. 2016; Knaus et al. 2020). In North America, these clonal lines would emerge by migration from a sexually reproducing population, potentially located in central Mexico (Knaus et al. 2016). Asexual reproduction patterns have also been described for *Ph. pluvialis* in New Zealand (Tabima et al. 2020) and *Ph. rubi* (Tabima et al. 2018). Similarly, the genetic structure of Australian *Plasmopara viticola*, responsible for grape downy mildew, also showed patterns of clonality while suggesting a single introduction in Western Australia (Taylor et al. 2019).

Genetic studies of *Peronospora* and *Pseudoperonospora* spp. are less abundant. Genetic study of *Ps. cubensis* North American populations revealed asexual reproduction mode, with important migration of new genotypes within the growing season while a high level of inbreeding was observed within the entire populations (Quesada-Ocampo et al. 2012; Summers et al. 2015a; Naegele et al. 2016). Similar findings were obtained for *Ps. humuli* (Gent et al. 2019). For *Peronospora effusa*, although epidemics seemed to be dominated by few clonal lineages, there was sufficient genetic diversity to suggest a mixed mode of reproduction (Lyon et al. 2016). For *P.*

tabacina, an important linkage disequilibrium showed that except for Australia and Mediterranean Europe, the populations contained partially clonal subpopulations, while the latter two reproduced sexually (Nowicki et al. 2021)

2.5 A favorable environment

2.5.1 Spore germination and infection

Infection by *P. destructor* begins with the germination of sporangia on the leaf surface, followed by germ tube penetration, primarily through stomata. As the germ tube elongates, protoplasm mass at the end of the germ tube and the spore is emptied of protoplasm, resulting in the formation of a round to elongated appressorium followed by the formation of a vesicle in the substomatal cavity (Palti 1989; Yarwood 1943). A period of continuous leaf wetness is essential for appressoria formation. However, temperature plays an almost equally important role. Appressoria formation occurs at temperatures between 3.5 and 26°C. However, when the temperature is between 10 and 18°C, the formation of appressoria is maximum after 2-3h while it takes 6h for the same result at a temperature of 22°C (Hildebrand and Sutton 1984a).

Following appressoria formation, hyphae develop intercellularly, mainly in the parenchyma of the palisade tissue. When foliage wetness conditions are conductive, host penetration can occur in less than 3h at temperatures between 6 and 18°C, whereas it can take between 6 and 10h at 26°C (Hildebrand and Sutton 1984c). Once the germ tube has penetrated the host plant, the mycelium can progress in the plant at a rate of 3 to 7 mm per day (Yarwood 1943; Palti 1989). Theoretically, it takes 7 to 10 days for the first symptoms to appear following infection, but symptoms usually appear between 10 to 17 days for new sporulation to appear on the leaf surface (Yarwood 1943; Palti 1989).

2.5.2 Sporulation

In *P. destructor*, sporangia production is strongly influenced by the interaction between relative humidity and day and night periods. Initiation of sporulation occurs only during the night when the onset of the high relative humidity period (>95%) begins rapidly after nightfall (Hildebrand and Sutton 1984b; Sutton and Hildebrand 1985). If the onset of high humidity begins less than 2h before the onset of darkness, sporangia production will be abundant, and the potential for sporangia production will decrease rapidly to zero if the high humidity period begins more than 4-5h after

nightfall (Hildebrand and Sutton 1984b; Sutton and Hildebrand 1985). It is important to mention that sporulation does not occur during a period of continuous light or darkness (Palti 1989). Sporangia production is inhibited when a film of water accumulates on the surface of the foliage during the night, either because of rain or too early a morning dew (Hildebrand and Sutton 1982).

Although secondary, temperature plays an important role in sporulation. For example, at a temperature of 6°C, even if the onset of the high humidity period starts less than 2 hours before nightfall, sporulation will be sparse, whereas, at temperatures between 10 and 22°C, sporulation will be abundant. In fact, the later the period of high relative humidity after daybreak, the narrower the effective temperature range. Thus, when moisture occurs 4-5h after dark, sporulation occurs only at temperatures between 14 and 18°C (Hildebrand and Sutton 1984b; Sutton and Hildebrand 1985). Temperature is also important the day before; sporangia production requires temperatures below 24°C, while temperatures above 28°C the day before inhibiting sporulation (Hildebrand and Sutton 1984c, a; Sutton and Hildebrand 1985).

2.5.3 Prediction of disease development

The seminal work conducted by Sutton, Hildebrand, and Jesperson in the 1980s, led to the development of downcast, a first rule-based model for predicting *P. destructor* sporulation events, a negative prognosis (Hildebrand and Sutton 1982, 1984c, a, b; Sutton and Hildebrand 1985; Jesperson and Sutton 1987). This model, although relatively simple, is still used in many onions growing regions, including Quebec and Ontario. The model was improved by de Visser, who adapted DownCast for the conditions encountered in Holland (De Visser 1998, 2001). In another attempt to improve the prediction of onion downy mildew, the ONIMIL model was developed in Italy (Battilani et al. 1996). This model is also based on meteorological data, but instead of providing a binary response, Onimil provides a quantitative output of infection risk. Later, the ZWIPERO model was developed by the German Meteorological Service. ZWIPERO determines the risk of sporulation and infection quantitatively based on actual and forecasted weather data (temperature, relative humidity, leaf moisture, and precipitation) (Friedrich et al. 2003). The most recent model, MILIONCAST, was developed in the UK following trials under controlled conditions and then empirically adjusted (Gilles et al. 2004). The authors also improved the accuracy of the ONIMIL and De Visser's Downcast, notably by lowering the minimum relative humidity for sporulation to 92% (Gilles et al. 2004).

2.5.4 Sporangia survival

Survival of mature sporangia is critical for the organism to colonize other plants, fields, or regions. Survival of sporangia is decisive in determining the length of time that spores remain viable during dispersal and thus the effective dispersal distance. The factors affecting sporangia survival have even been used recently to develop a model for estimating the risks of spread of *Phytophthora infestans,* another airborne oomycete (Skelsey et al. 2018). In the case of *P. destructor*, relative humidity (RH) and temperature play an important role in sporangia survival. In trials carried out under controlled conditions, Bashi and Aylor (1983) showed that the germination rate of sporangia after 72h exposure to 10°C was 60% when RH was over 53% and less than 20% when RH was 33%. At 25°C, the germination rate decreased to 20% after 48h, even when RH was above 75%. At 35°C, the germination rate dropped to 20% within 6h. Despite the importance of these factors, solar radiation would play an even more important role. When sporangia were exposed for a period of 6 hours to radiation of 70-280, 280-630, and 630-930 W/m2, the germination rate was 68, 5, and 0%. This effect of solar radiation is more difficult to demonstrate under environmental conditions. Using plant traps, Hildebrand and Sutton (1984a) found that sporangia could survive under sunny or cloudy, hot or cool, wet or dry conditions. In this study, dew, specifically the rate of dew formation, had the greatest influence on survival.

2.5.5 Dispersal

The sporangiophores of *P. destructor* have the particularity of coiling on themselves, which creates a tension allowing a certain discharge of the sporangia (Leach et al. 1982; Leach 1982). The onset of this spore ejection is mainly triggered by a decrease in relative humidity from saturation. Short exposure to infrared radiation (~3695µW/cm^2) at low relative humidity (~45%) can also trigger sporangia discharge (Leach et al. 1982). Airflow causing vibration can also trigger sporangia discharge. In their work, Leach et al (1982) demonstrated that an airflow of 0.5 m/s was sufficient to prompt sporangia discharge. This characteristic may be of great importance in onion fields exposed to various vibration sources (wind, irrigation drop, vibrations caused by the movement of machinery, etc.) and could be used as a variable in predictive models.

When the sporangia are discharged, they are transported over more or less long distances by the wind. The dispersal of *P. destructor* sporangia has been little studied, and it is necessary to go back to 1938 to find a study on the dissemination of *P. destructor* spores. In this study, the authors

collected samples at elevations from 15 to 450m. In this study, sporangia were collected at more than 250m, and nearly 50% of the sporangia collected at this height were viable and capable of germination (Newhall 1938). Horizontal dispersal was not studied for *P. destructor* specifically.

2.5.6 Disease carryover and off-season survival

Overwintering and disease carryover is probably one of the least well-documented aspects of the disease. Like most Peronosporales, *P. destructor* forms oospores. However, the conditions favoring their development, the mating system, and the general role that oospores would play in the epidemiology of the pathogen is still not fully understood. The patterns of off-season survival and the mode of carryover from one crop to the next can vary depending on the region and climate. This off-season survival consists of overwintering in some areas with cold winters, such as in the Northeast of North America, or surviving the summer in some areas with mild winters and dry, hot summers such as California (Yarwood 1943). In addition, off-season survival patterns could change as the pathogen adapts to the changing climate. The different modes of off-season survival could be carryover as oospores in soils and crop debris; carryover as mycelium in bulbs, seeds, or soil; and seasonal reintroduction of sporangia by wind or other organisms from an infected area to an uninfected one.

It has long been known that *P. destructor* can produce oospores in large quantities. However, it was soon reported that these oospores rarely germinate under laboratory conditions (Blackwell 1935; McKay 1935, 1937). In 1930, after finding an onion crop heavily affected by *P. destructor* and showing foliage with "enormous numbers" of oospores, McKay (1935) designed and initiated an experiment on oospore germination. First, he found that the oogonium collapsing around the oospores was surprisingly persistent and observed that this formed a 2-3µm thick protective layer around the already thick oospore wall. The second important finding from this study was that no changes could be observed in the oospores, and, consistently, germination could not be observed before the fourth year (McKay 1935). Later it was hypothesized that oospore germination depends on maturation, which results in a reduction in cell wall thickness and an increase in cell wall permeability, and on a time factor (Blackwell 1935). This hypothesis was confirmed insofar as an increase in germination rate was observed as a function of time, although only 2% germination was observed on oospores four to six years old, and that the addition of potassium permanganate increased the germination rate to more than 60% for oospores of the same age (McKay 1937). It

was also found that the germination rate of oospores continues to increase with time and that oospores remain viable for at least 25 years (McKay 1957). Despite this ability to produce abundant, long-lived oospores, none were able to infect onions from oospores under experimental conditions, raising questions about the role of oospores as a source of primary inoculum (Palti 1989).

Apart from the time factor and the need for oospores maturation, the climatic factors influencing the production, germination, and survival of oospores are not well documented in peronosporales. For many species, oospore production occurs when the amount of resources decreases, that is when the plant collapses following infection (Judelson 2008). However, it was shown that oospore formation in *P. cubensis* could occur when both types of mating were present at 12.5°C to 20°C under a moisture-saturated atmosphere, with optimum temperature between 15°C and 20°C (Cohen et al. 2011; Cohen and Rubin 2012). Oospores were not produced at 25°C. Similarly, for *P. viciae* f.sp. *pisi*, more oospores were produced at 15-20°C compared to temperatures of 5 and 10°C (Van Der Gaag and Frinking 1996). Temperature is also a major factor in oospore infection. It has been reported that oospore germination was optimal at temperatures of 15-20°C for *P. cubensis* (Cohen et al. 2011), 16°C for *P. arborescens* (Montes-Borrego et al. 2009) and 20°C for *Plasmopara obducens* (Shishkoff 2019). For the latter, it was observed that instead of continuous incubation temperatures, fluctuating temperatures (between -10°C and 0°C or between 0°C and 10°C) could also induce oospore germination (Shishkoff 2019). The study of the factors influencing the survival and infectivity of oospores is relatively difficult since the latter can be very resistant and require a long maturation period, as it is the case for *P. destructor* but also for other species like *P. humuli* (Gent et al. 2017) or *P. tabacina* (LaMondia and Aylor 2001). To overcome this limitation, some have shifted to the use of observational data combined with the use of multivariate statistics to study disease carryover. For *P. infestans*, for example, it has been shown that a high level of late blight at the end of the year influences disease development the following season (Zwankhuizen and Zadoks 2002). Similarly, it was also suggested that earlier late blight epidemics could be related to soilborne inoculum from the 1990s in Finland, especially due to climate change and lack of rotation (Hannukkala et al. 2007).

The possibility that the carryover of onion downy mildew may be through dormant mycelium in onion plants was established in Ireland in the 1920s (Murphy 1921) and subsequently confirmed in different contexts (Hildebrand and Sutton 1980; Yarwood 1943). It was demonstrated that

artificially inoculated onion sets could remain viable at temperatures as low as 1-3°C (Hildebrand and Sutton 1980). At these temperatures, 95%, 91%, and 73% of the inoculated plants showed symptoms of onion downy mildew after 0, 47, and 166 days. Even after six months of storage at 1-3°C, 20% to 50% of the plants showed symptoms. In contrast, only 40% of the plants maintained at a temperature of 20-22°C for a maximum of 59 days showed onion downy mildew symptoms (Hildebrand and Sutton 1980).

The role of long-distance dispersal in downy mildews has been thoroughly studied in tobacco (LaMondia and Aylor 2001), cucurbits (Ojiambo and Holmes 2010) and to a lesser extent, spinach (Choudhury et al. 2016). In a study of tobacco blue mold caused by *P. tabacina*, LaMondia and Aylor (2001) reported that the disease had occurred in Connecticut only seven times in 44 years and that, given the long periods between outbreaks, long-distance transport was a rare event. However, given the very large number of sporangia that *P. tabacina* could produce, it was concluded that a local source could pose an equivalent risk to a source 700 km away when the weather is cloudy, and the sporangia are not exposed to UV radiation (Aylor et al. 1982). A good indicator of long-distance transport is the northward progression of a disease front (Aylor and Irwin 1999). This south-north progression can be expressed as time-space trajectories where the slope of this trajectory represents the progression rate of the migration front (Aylor 1999; LaMondia and Aylor 2001). This phenomenon has been well documented for *P. cubensis*, while the 2008 and 2009 outbreaks were analyzed as a part of the ipmPIPE program. In this study, it was shown that cucumber late blight outbreaks progressed at a rate between 9.2 and 10.5 km per day (Ojiambo and Holmes 2010). Disease outbreaks were spatially aggregated with a range of spatial autocorrelation of up to 1000 km (Ojiambo and Holmes 2010). Patterns of long-distance dispersal were also hypothesized in spinach downy mildew caused by *P. effusa* (Choudhury et al. 2016).

Connecting statement for chapter 3

As mentioned in the previous section, long-distance dispersal is an essential component of the carryover of airborne pathogens. However, the transport of spores over short distances also plays a crucial role once epidemics begin. The significance of knowing the size of airborne plant pathogen populations is documented, but several technical challenges limit the development of agricultural aerobiology. Given the importance of both long and short distances dispersal in plant disease epidemiology, chapter 3 is devoted to reviewing the uses of aerobiological data for biovigilance, surveillance, or monitoring purposes. This review attempts to discuss essential factors to consider when establishing airborne inoculum monitoring networks and interpreting aerobiological data.

This review was submitted and is now published in Agronomy for Sustainable Development. Among the top five journals in agronomy, this journal publishes critical evaluation of emerging topics, sustainable agricultural systems, and decision support tools. Moreover, the approaches presented in this journal must be applicable at the field, farm, landscape, and global scales. This manuscript is now published as:

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3 Monitoring airborne inoculum for improved plant disease management. A review

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3.1 Abstract

Global demand for pesticide-free food products is increasing rapidly. Crops of all types are, however, under constant threat from various plant pathogens. To achieve adequate control with minimal pesticide use, close monitoring is imperative. Many plant pathogens spread through the air, so the atmosphere is composed of a wide variety of plant pathogenic and non-plant pathogenic organisms, in particular in agricultural environments. Aerobiology is the science that studies airborne microorganisms and their distribution, especially as agents of infection. Although this discipline has existed for decades, the development of new molecular technologies is contributing to an increase in the use of aerobiological data for several purposes, from day-to-day monitoring to improving our understanding of pathosystems. Although the importance of knowing the size and composition of plant pathogen populations present in the air is recognized, technical constraints hinder the development of agricultural aerobiology.

Here we review the application of spore sampling systems in agriculture and discuss the main considerations underlying the implementation of airborne inoculum monitoring. The results of this literature review confirm that the use of aerobiological data to study the escape of inoculum from a source and its role in the development of diseases is well mastered, but point at a lack of knowledge to proceed with the deployment of these systems at the landscape scale. Thus, we conclude that airborne inoculum surveillance networks are still in their early stages and although more and more initiatives are emerging, research must be conducted primarily to integrate evolving technologies and improve the access, analysis, interpretation and sharing of data. These tools are needed to estimate short- and medium-term risks, identify the most appropriate control measures with the lowest environmental risk, develop indicators to document the effects of climate change and monitor the evolution of new genotypes at multiple scales.

3.2 Introduction

Air is the means of transport for many plant pathogens, some of which can travel thousands of kilometers while maintaining their viability and ability to cause new epidemics (Stakman and Christensen 1946). Many plant pathogenic fungi are remarkably well adapted to airborne spread. The fact that fungal spores are spread by air currents has been known for almost as long as the existence of the spores themselves. In 1729, Micheli published the results of research on spore production and demonstrated that 'clouds' of spores could be released into the air. The atmosphere in agricultural environments is therefore full of particles ranging in size from 0.1 µm for viruses to 100 µm for pollens. Monitoring spores and other particles in the air requires a specific field of scientific expertise, which is called aerobiology.

Aerobiology has developed to a large extent in response to the need to know the quality of air in buildings and of pollen responsible for allergies in humans. Philip Herries Gregory is the pioneer of aerobiology in agriculture. By the late 1930s, he was already studying the movement of fungal spores in the air, including those of *Phytophthora infestans* which is responsible for potato late blight (Gregory 1945, 1973). Dr. Gregory initiated the aerobiology research program at Rothamsted Research (formerly the Rothamsted Experimental Station) in England, which for decades has contributed and continues to contribute to the advancement of knowledge in aerobiology. Contributions includes the study of processes involved in the airborne spread of plant pathogens (Williams et al. 2001; McCartney et al. 1997), spores disseminated in aerosols (Fitt et al. 1985) and the use of information on airborne inoculum as risk indicators (Rogers et al. 2009; West et al. 2008). Since Dr. Gregory's early work, the use of spore samplers for the study of airborne inoculum has continued to develop (Lacey 1996) for diseases that affect: cereal (Chamecki et al. 2012; Hellin et al. 2018; Hemmati et al 2001; Morais et al. 2015; Paulitz et al. 1998), industrial crops (Bashan et al., 1991; Alderman, 1993), field crops (Chawda and Rajasab 1994; Fitt et al. 1985; Reich et al. 2016), fruit crops (Charest et al. 2002; Carisse et al. 2009a; Aylor 1995) and vegetables (Carisse et al. 2005; Carisse et al. 2009b).

Although the importance of considering airborne inoculum seems obvious from a disease management perspective, spores of plant pathogenic fungi are very small, many without a distinctive color or shape, and therefore very difficult to identify and count. As a result, the difficulty of counting spores in air samples has long limited the use of information on airborne

inoculum in the management of airborne diseases. Over the years, different counting methods have been assessed, but methods using polymerase chain reaction (PCR) have really made it possible to use spore samplers at large scale with standardized counts (Carisse et al. 2009b; Falacy et al. 2007; West et al. 2008). Aerobiological research conducted worldwide during the last two decades represent a turning point as they demonstrated the possibility of using information on airborne inoculum to improve disease management. Published original research articles (Carisse et al. 2012; Van der Heyden et al. 2012; Rogers et al. 2009; West et al. 2008), books (Aylor 2017; Gregory 1959) and reviews (West and Kimber 2015; Mahaffee and Stoll 2016; West et al. 2008; Jackson and Bayliss 2011) covered theoretical and applied, general or specific aspects of aerobiology and airborne spore sampling. In this review, we wish not to repeat former publications, but to discuss the state of knowledge related to the implementation of networks of airborne fungal spore monitoring.

3.3 Purposes of sampling airborne fungal spores

The primary purpose of aerobiology is to improve our understanding of the epidemiology of airborne diseases at different temporal and spatial scales. Aerobiological data are used in a wide range of studies, several examples of which are provided in Table 1. Regardless of their nature, aerobiological data can be used to model disease development and to evaluate different types of disease forecasting systems (Fall et al., 2015b). In comparative epidemiology, airborne inoculum progress curves can be used to study the role of airborne inoculum on disease development in relation to climatic conditions, production systems, cultivars, control method, etc. (Carisse et al. 2014). In addition, when aerobiological data are combined with genetic analyzes, it become possible to study fungal community and ecology of different pathogen genotypes including those related to fungicide resistance or aggressiveness (Fraaije et al 2005; Hellin et al 2020; Nicolaisen et al 2017).

Sampling air for fungal spores is also motivated by the purposes of biovigilance, surveillance and monitoring. In the case of biovigilance, it can be used to measure the impacts of climate change and production practices or systems on populations of plant pathogens and beneficial agents, or to study the dispersion of emerging pathogens colonizing new agricultural environments, the emergence of new species or the genetic diversity of plant pathogens (Chen et al. 2018). With a purpose of surveillance, air is sampled in a particular region to measure a disease risk for plant

pathogens that are present but cause sporadic or variable levels of damage. Also, it is important to monitor periodically introduced plant pathogens originating from areas further away, to detect their arrival in a specific area and react effectively. Potato late blight (*P. infestans*) and cucumber downy mildew (*Pseudoperonospora cubensis*) are examples of this type of plant diseases (Cohen and Rotem 1971; Rahman et al. 2020; Fall et al. 2015). Surveillance typically takes place over the long term, ideally at the same sites to facilitate year-to-year comparison. For endemic plant pathogens and for those that migrate via air, transplants or seeds, a key element of disease management is the ability to determine when secondary inoculum is produced and how much is produced. In the case of monitoring, air sampling is combined with agronomic and meteorological data, to identify risks and optimize phytosanitary measures (Carisse et al. 2012; Van der Heyden et al. 2012). Airborne inoculum monitoring activities typically take place at a high frequency and at the spatial scale of the field, farm or region, in order to allow day-to-day disease management decision making. Aerobiological data can therefore be used to improve forecasting systems and optimize within seasonal decision-making regarding fungicide applications.

3.4 Trajectory of fungal spores in the air

As a scientific discipline, aerobiology requires a good understanding of the phenomena involved, namely the production of inoculum (spores), the detachment of spores from their production site, their exit from the plant canopy, their dispersion (transport) in the air over long or short distances, the viability of the spores, and their deposition at new infection sites. The primary inoculum source varies depending on the plant pathogenic fungus and the production system. The sources may be seeds, transplants, volunteer plants, crop or warehouse debris, survival structures (e.g. sclerotia, cleistothecia, oospores), or resting growth structures (mycelium). Depending on the primary inoculum source, the first inoculum production sites are distributed over larger or smaller areas or confined to specific locations with a random or aggregated spatial distribution. For secondary inoculum, the number of production sites generally increases with disease progression.

Plant pathogenic fungi have different spore-release mechanisms, passive or active (Ingold 1971). For many fungal pathogens, spores are released only when they are shaken, splashed, or blown from their mycelia or sporophores; sometimes removed by insects. These spores released mechanisms are considered as passive. For example, most conidia are dislodged from their conidiophores and are dispersed only when exposed to specific physical disturbance such as wind

or rain droplets (Fitt et al. 1985). Hence, fungi with passive spore release mechanisms often sporulate on parts of plants exposed to the wind (Ingold 1999). Other fungi have developed active spore release mechanisms that allow spores to escape from the calm air zone regardless of wind exposure (Aylor 1995). Some spores are ejected from fruiting structures (eg pseudothecia or perithecia) as a result of increased pressure inside the structure. This pressure can be osmotic or caused by the hydrolysis of substances such as glycogen. For other fungi, the projection of the spores is obtained following the production of a droplet of exudate which suddenly changes position while moving towards the point of attachment of the spore, causing a tension which releases the spore. (Ingold 1971). Some spore release mechanisms are somehow between passive and active for example, spores released in response to the twisting of their sporophores as they desiccate in dry air. Regardless of the mechanism, the exit of spores from the plant cover (laminar zone of calm air) is essential for spores to escape, disperse in the turbulent zone and travel over short or long distances (Gregory 1973; Aylor 2017).

Once spores are released into the air, they disperse, and their concentration decreases as the distance from the release point increases (Aylor 2017; Gregory 1973). Spore dispersion within and outside the plant canopy is difficult to measure since air movement affects the release, dispersion and deposition of spores at new infection sites (Legg 1983). In the context of airborne inoculum monitoring, spore concentration is generally measured at the exit from the plant canopy, during the dispersion (transport) or deposition phase, the latter being more often used when sampling is conducted for disease risk estimation.

Aerobiology also includes the study of the influence of weather conditions, landscape architecture, plant cover and cultural practices on all stages of trajectory of spores in the air, from spore production to spore deposition at new infection sites. Aerobiology is multidisciplinary in that knowledge of plant pathology, disease epidemiology, ecology of plant pathogens, mycology, molecular biology, mathematics, statistics, and modelling is required to properly measure and interpret aerobiological data.

3.5 Types of samplers

Spores can be collected with different types of samplers, depending on the purpose of the study and each type of sampler has its advantages and disadvantages. A number of factors should be considered when choosing the most appropriate sampler: its efficiency, the sampling volume, the

length of the sampling period, the amount of time the sampler can stay in the field, the ease of manipulating samples, the size of the particles to be sampled and the frequency of monitoring. The characteristics of each type of samplers were described in depth by West and Kimber (2015), so we only cover the basics in this section (Table 2). In addition, examples of samplers used in different studies are provided in relation to the pursued objectives (Table 1).

The first category of devices used to collect spores can be described as passive samplers. Their operation is based on the deposition of spores on an adhesive surface (i.e., petri dish, microscope slide), through a slide or a filter (West and Kimber 2015). The simplest example consists of installing microscope slides coated with an adhesive substance (e.g., silicone grease, petroleum jelly) on a vertical, horizontal or inclined support (Fig. 1A). The obvious advantages of this type of device are its low cost and the direct mounting of the slides on a microscope. However, it does not allow the calculation of spore concentrations in the air (i.e., numbers of spores per m^3 of air) because the amount of air sampled is unknown. Moreover, the reading of the microscope slides can be difficult and time consuming depending on the thickness of the silicone layer used and the amount of debris present on the sampling surface. However, this type of device can be useful for monitoring in areas that are large or difficult to access, where resources are limited, or if only the presence or absence of a given spore species is needed.

Funnel-type samplers also belong to the category of passive samplers. They consist of a vertical cylinder topped by a funnel, at the base of which is installed a filter where are collected the spores captured by the sampler. This type of sampler is suitable for spores travelling long distances and deposited during rainfall events, such as *Phakopsora pachyrhizi* (Asian soybean rust) (Isard et al. 2011), or for spores dispersed by splashes, such as *Fusarium graminearum* (Paul et al. 2004). It is possible to quantify the inoculum per volume of water sampled when a weather station is installed nearby or when a rain gauge is installed directly on such a sampler.

The third type of passive sampler consists of a cylinder ending in a fin (or aileron) installed on a ball joint, so that it is facing the wind constantly. Spores are collected in a filter installed at the bottom of the cylinder. This type of device has the advantage of being inexpensive, which allows for large-scale deployment, but it hardly provides the inoculum concentration. In addition, counting spores on the filter can be laborious as they cannot be counted directly without first being dislodged from the filter mesh. This type of device is used in particular for large-scale monitoring,

when binary data (presence/absence) is sufficient. This type of sampler has been used to monitor the progression of Asian soybean rust (*P. pachyrhizi*) throughout North America, from Florida to Canada (von Qualen and Yang 2006). This kind of device is also used to monitor potato late blight (*P. infestans*) in Ontario (Eugenia Banks, personal communication) (Fig. 1D).

Active (or volumetric) spore samplers are much more widely used, mainly because they allow the calculation of airborne spore concentrations (in spores/m^3 of air). As opposed to passive samplers, these samplers are referred as active because the sampled particles are collected actively and use a collection mechanism (a rotor, a pump or an electrostatic force field). They can be classified into four main categories: impaction samplers, cyclone samplers, ionic samplers and virtual impactors (West et al. 2008; West and Kimber 2015). The first can involve impaction on Petri dishes containing a culture medium, on adhesive surfaces (tape, slides, rods) or in a liquid medium (Lacey and West 2006) (Fig. 1B-C). Cyclone samplers collect spores directly into a microcentrifuge tube to facilitate sample manipulation (Fig. 1E). Ionic samplers capture spores on an electron microscope electrode using a powerful electric field. Spores can therefore be counted directly using a scanning electron microscope. The virtual impactor can sample up to 850 liter of air per min (Limpert et al. 1999). It allows to separate the sampled particles according to their size into two air currents. Smaller particles with low inertia are deflected with the main flow while larger particles with greater inertia are directed to the collection device (Schwarzbach 1979).

In general, rotating-arm-type impaction samplers and 7-day volumetric spore samplers are well suited for monitoring fungi and oomycetes spores larger than 10 µm. These are the two most commonly used types of samplers in agriculture (Carisse et al. 2005; 2007; 2008; 2009b; 2012; 2013; Choudhury et al. 2016; Fall et al. 2015a; 2015b; 2015c; Friedrich et al. 2003; Hellin et al. 2018; Klosterman et al. 2014b; Kunjeti et al. 2016; Reich et al. 2016; Van der Heyden et al. 2012b; 2014b). For small particles, a filter sampler (e.g., Button Aerosol Sampler) or a wet cyclone (e.g., Bertin Coriolis sampler) may be more appropriate.

3.6 Sampling frequency and duration

One of the most important factors for the monitoring of airborne spores is undoubtedly the periodicity at which spores are released into the air. This characteristic dictates the best time of day for sampling, its frequency and duration. The term “sporulation” generally includes the production of spores and their dispersion in the air. For many species, spore production takes place

at night, while spore dispersion usually takes place during the day. Periodicity has been studied more particularly since 1953, for *Alternaria sp.*, *Cladosporium spp.*, *Ustilago spp.*, *Erysiphe spp.*, *Polythrincium trifolii* and *P. infestans* (Hirst 1953). In Hirst's pioneer work, it is suggested that spore emission is generally highest around noon (closer to 11h00 for *P. infestans* and *P. trifolii* and closer to 13h00 for *Alternaria spp.*, *Cladosporium spp.*, *Ustilago spp.* and *Erysiphe spp.*) (Hirst 1953).

Subsequently, with the advancement of technical and practical knowledge, periodicity patterns were more accurately described for different pathogens. For example, a specific circadian dispersion pattern was described for *P. infestans* (Aylor et al. 2001). Although the results are essentially consistent with Hirst's work (i.e., emission peaks around 11h00), Aylor et al. (2001) found that spores could be captured between 8h00 and 20h00. For their part, Hildebrand and Sutton (1982) reported that, although the first sporangia of *Peronospora destructor* could be captured on average 1.5 hours after sunrise, the time of emission peaks could vary with relative air humidity, drying of foliage, and wind speed. In this study, spores were collected mainly under winds between 0.3 and 1.0 m/s and emission peaks measured between 10h00 and 14h00 (Hildebrand and Sutton 1982). Similarly, the airborne concentrations of *Bremia lactucae* sporangia, which are responsible for lettuce downy mildew, are variable, with emission peaks between 8h00 and 14h00 (Fig. 2) (Fall et al. 2015a; Fall et al. 2016).

Whereas some pathogenic fungi have clear periodicity patterns, others are less characteristic. In carrot cultivation, for example, the emission patterns of *Alternaria dauci* spores tend to follow a normal curve with a nearly constant spore concentration between 10h00 and 16h00 (Strandberg 1977). Similarly, a study on the inoculum sources of *Alternaria spp.* suggested that peaks of spores emission ranged from 9h00 to 17h00 (Fernández-Rodríguez et al. 2015). This variation in spores periodicity patterns was also observed for other airborne pathogen like *Botrytis cinerea* and *B. fabae*, which were reported to be between 10h00 and 14h00 with occasional peaks between 14h00 and 17h00 in raspberry (Jarvis 1962), between 8h00 and 14h00 in strawberry and bean (Blanco et al. 2006; Fitt et al. 1985).

In order to adjust for this variation in circadian patterns of sporulation, several studies suggest splitting the sampling period. In onions, for example, spore sampling for *B. squamosa* was initially set for a fixed period of 2 hours between 10h00 and 12h00 (Carisse et al. 2012; Carisse et al. 2005;

Carisse et al. 2007, 2008; Carisse et al. 2009b; Van der Heyden et al. 2012), but experiences has shown the importance of splitting the sampling period, which is now distributed between 8h00 and 14h00, 50% of the time (Carisse and Van der Heyden 2017). In a study conducted in Quebec to characterize the spatial distribution patterns of airborne conidia of *Podosphaera aphanis* in strawberries, the sampling period was fractionated so that effective sampling was carried out 30% of the time between 10h00 and 15h00 (Van der Heyden et al. 2014b). Similarly, in a study conducted in New Brunswick, sampling of airborne inoculum from *P. infestans* was performed for 50% of the time between 6h00 and 15h00 (Fall et al. 2015b). This sampling range was also used for monitoring *B. lactucae* in lettuce (Fall et al. 2015a). Hence, the sampling period and frequency within the sampling period depend on the period and duration of the spore release as well as how fast the device sampling surface (e.g., rods, tape, etc.) become saturated. To circumvent the need to split the sampling period, others have chosen to use the 7-day sampler, which enables to obtain hourly or daily data (Hellin et al. 2018; Reich et al. 2009; Rogers et al. 2009.

3.7 Sampling height

Once the sampling period has been determined, the height at which the spore sampler is placed becomes one of the most important criteria to consider. The height at which a sampler should be installed depends largely on the objective of the study and in the best-case scenario, a tower with samplers installed at different heights should be recommended (Aylor, 2017). However, it is often difficult to install such towers in the field, with the exception of experimental plots. Hence, a general rule of thumb might be to place the sampler above the canopy for the measurement of local inoculum, while the measurement of incoming inoculum would require the samplers to be placed higher, a few meters above the ground. Several examples are provided in Table 1, and more details are given below for selected examples.

The effect of the sampler height on spore concentration measures made using impaction samplers has been studied for some plant pathogens (Aylor 1995; Aylor and Taylor 1983). The objective of these studies includes estimating the proportion of spores escaping from the canopy from a point source. This is achieved by installing spore samplers at regular distances above a source and counting their contents at regular intervals to characterize vertical dispersion gradients. In a 1981 test, concentrations of *P. tabaccina* spores, for example, ranged from 384.6 spores/m^3 of air at 0.75 m above the ground to 0.9 spores/m^3 of air at 4 m above the ground (Aylor and Taylor

1983). On average, concentrations measured at 4 m above the ground represented only 12% of the spore concentrations measured at 0.75 m above the ground (Aylor and Taylor 1983). In the case of *Venturia inaequalis*, responsible for apple scab, spore concentration also decreased rapidly with height, with concentrations measured at 3 m corresponding to 6% of the concentrations measured at 0.15 m above the ground (Aylor 1995). The case of *P. infestans* has been extensively studied in this regard. In tests conducted in 1999 and 2000 in the State of New York, the vertical distribution of *P. infestans* spores showed that spore concentration decreased significantly with height, from nearly 6,000 spores/m^3 of air at 0.75 m above ground to less than 10 spores/m^3 of air at 3 m above ground in 1999 and from 2,000 to 10 spores/m^3 of air in 2000 (Aylor et al. 2001). In other words, for crops with low vegetation cover, the concentration of inoculum from a point source in the field quickly fades and the probability of detection is almost null from 3 m above the ground.

For a point source inoculum, the effect of the plume of spores escaping from the canopy dilutes rapidly, and the probability of capturing spores decreases with distance, vertically and horizontally (Severns et al. 2018). The effect of sampling height on the concentration of spores collected from multiple sources is much less documented. However, under field conditions, it can be assumed that there are multiple sources of inoculum and the horizontal flow of spores in the wind direction is equal to the cumulative concentration of spores escaping from the canopy from each source, between the edge of the field or the first source and the location of the sampler (Aylor, 2017). Thus, at the field scale, spore concentrations increase in the prevailing wind direction based on the distance from the farthest source and the number of sources between the farthest source and the sampler location (Fig. 3). Hence, it is possible to optimize the position of the spore sampler according to the distance from the edge of the field or the number of inoculum point sources in the direction of the prevailing winds (Fig. 3) (Aylor 1995; Chamecki et al. 2011).

Knowledge of vertical distribution patterns can be exploited to the user's advantage, particularly to distinguish inoculum originating from within the field versus from outside the field (incoming inoculum). For example, samplers that are part of an airborne inoculum monitoring network dedicated to monitoring the local *Botrytis squamosa* inoculum, responsible for botrytis leaf blight, are deployed at a height of 1 m early in the season and their height is adjusted according to plant growth (Carisse et al. 2012; Carisse and Van der Heyden 2017; Van der Heyden et al. 2012b). For strawberry powdery mildew, samplers installed 1 m above the ground were found to be more representative of a plot than those installed at a height of 0.35 m (Van der Heyden et al.

2014b). In research conducted in California on spinach and lettuce, spore samplers used to monitor downy mildew were installed at 0.53 m above the ground, for practical reasons (Choudhury et al. 2016; Klosterman et al. 2014b; Kunjeti et al. 2016). In Belgium, the relationship between airborne concentrations of *Fusarium graminearum* spores and concentrations of deoxynivalenol (DON) was characterized using spore samplers installed 1 m above the ground (Hellin et al. 2018). Concentrations of grape powdery mildew in Oregon and Quebec and *B. cinerea* in strawberries, are monitored using spore samplers placed at heights of about 1 m (Carisse et al. 2009a; 2014; Thiessen et al. 2016). In a different context, spore samplers were also used between 2009 and 2012 for the implementation of a monitoring network for the inoculum of *P. infestans* in potatoes, aimed at characterizing regional dispersion patterns and identifying exogenous inoculum sources (Fall et al. 2015b). In this context, the samplers were installed at 2.9 m high to collect spores mostly coming from outside the fields.

3.8 Spatial distribution patterns and dispersal gradients

During the establishment of a spore sampling network, it is important to know as much as possible about the spatial distribution of the pathogen airborne spore concentrations to be monitored. More precisely, the study of spatial distribution patterns allows one to deduce the nature of the dispersion processes of the pathogens or diseases of interest and hence, improve the sampling. Does the pathogen spread over short distances from one plant to its neighbor? Or does it travel over long distances from one field to another, or even from one region to another? In other words, learning the spatial scope of the mechanisms involved in inoculum dispersion processes for the monitored species is the aim. In an agricultural field or a plantation, diseased plants among the plants present can have three types of spatial distribution: regular, completely random and aggregated (Fig. 4).

The type of spatial distribution has an impact on the effectiveness of sampling strategies. In the case of traditional field scouting (symptom scouting), the probability of finding a diseased plant is higher when the distribution of the disease in the field is completely random, compared to an aggregated distribution (Madden and Hughes 1999; Mahaffee and Stoll 2016). The spatial distribution of symptoms in the field was characterized for different pathosystems, revealing distribution patterns that varied between pathosystems. For example, the spatial distribution of *Sclerotinia sclerotiorum* symptoms in bean cultivation is largely dominated by a completely random pattern, both in pods and foliage (Jones et al. 2011). Patterns for hop powdery mildew

(*Podosphaera macularis*) also follow a completely random distribution in general (Turechek and Mahaffee 2004). A study conducted in Quebec suggests a completely random spatial distribution pattern for strawberry powdery mildew for 72% of the sampling dates (van der Heyden et al. 2014b). In the case of field scouting, the sampling routes and number of samples can be adjusted to the spatial distribution, making this epidemiological characteristic a non-limiting factor.

Unlike field scouting for symptoms, air sampling is more likely to detect the onset of an epidemic if the distribution of inoculum sources is aggregated and the sampler is positioned to intercept the plume of spores emitted (Aylor and Irwin 1999; Mahaffee and Stoll 2016). A good knowledge of the distribution patterns is therefore important to develop a reliable inoculum monitoring network based on spore samplers. However, despite the importance of the spatial distribution patterns, specific to each pathogen, very few studies described these patterns of spatial distribution of airborne spores at the field and regional scales.

At the field or plot scale, one of the first studies describing spatial dispersion patterns for spores targeted apple scab, caused by *Venturia inaequalis*. The results of this study, conducted on 40 108-m^2 plots in Quebec, suggested a patchy aggregated distribution pattern for most sampling dates and a spatial autocorrelation range between 25 and 53 m (Charest et al. 2002). Similarly, the spatial distribution of *P. aphanis* spores in strawberries is characterized by an aggregated pattern with no distinct focus (Van der Heyden et al. 2014b). However, spatial distribution patterns vary with time and disease intensity. For example, for *B. squamosa* in onions, spore concentrations measured in small plots reveal random dispersion patterns at the beginning of the season or when the inoculum concentration is low and more aggregated patterns when inoculum concentrations are higher (Carisse et al. 2007).

For some pathogens, spatial distribution and dispersion patterns have been characterized at a larger scale (regional or national). Such knowledge is particularly relevant to predict the risks of development of an epidemic at the territory scale rather than the field scale. Potato late blight, caused by *P. infestans*, is probably the most documented in this regard. In New Brunswick, spatial distribution patterns of *P. infestans* spores have been characterized, and the results suggest a heterogeneous distribution with the increase in spore concentrations during the season (Fall et al. 2015b). In addition, the authors note that this information can be used to guide decision making with a limited number of spore samplers. Results from the same study also suggest that epidemics

of downy mildew are not very affected by the size of the initial spore load, and support monitoring of the inoculum throughout the production period. Spatial distribution patterns of *P. effusa* spores in spinach have been characterized as highly aggregated, with a short spatial autocorrelation range (5.6 m on average), in the Salinas Valley, California (Choudhury et al. 2016). In this study, however, spore samplers were installed 0.53 m above the ground, which possibly represents the plot better than the region.

The aggregation patterns observed at the field scale in the studies above are generally the result of auto-infection processes (Mundt 2009). The concepts of auto- and alloinfection are important in epidemiology because they are largely associated with the dispersion of pathogens. Both concepts were first introduced by Robinson (1976), who considered that autoinfection occurs when an infected plant or part of a plant (donor) contaminates the same plant or part of a plant (recipient), and conversely, that alloinfection occurs when the donor and recipient are different. Autoinfection therefore is a process that occurs over short distances compared to the alloinfection process (Mundt 2009; Zawolek and Zadoks 1992). In some studies, autoinfection is considered to be an infection resulting from propagules belonging to the same genotype, produced from the same foci of contiguous plants, whereas alloinfection is considered as an infection resulting from propagules produced from other genotypic units in the population (Mundt and Browning 1985; Zawolek and Zadoks 1992). Autoinfection and allonfection generally occur simultaneously, and their occurrence can be expressed as a proportion of the sum of infections. Thus, the ratio of allo- and autoinfection varies throughout an epidemic, the proportion of autoinfection being higher at the beginning. The disease tends to accumulate locally until a dispersion threshold is reached, beyond which transmission of the disease to another location becomes likely (Aylor 2017).

When a focus of infection has reached the dispersal threshold, it becomes a source of inoculum that emits over varying distances. Dispersal gradients from single point source were described for several plant pathogens. Some pathogens are disseminated over short distances from a single source. For example, Fusarium head blight seeds infection caused by *Gibberella zeae* was shown to decrease to 10% of the maximum within 5 to 22m from the focal center of an inoculated plot (Fernando et al. 1997; Paulitz et al. 1999), while spores of *Mycosphaerella fijiensis*, responsible for Black leaf streak disease, showed a gradient of up to 12.5 m (Rieux et al. 2014). Other plant diseases like *P. infestans* can be dispersed over several 100 m to up to 700 km (Severns et al. 2018). Dispersion rates are also influenced by the manner in which spores are released. In simple

terms, spores can be divided into two categories: type 1 and type 2 (Fig. 5). Type 1 spores, such as *Botrytis* spp. conidia, are passively released from their attachment structure (e.g., conidiophores) by turbulent and unstable winds (Aylor 2017). The wind speed required to detach and disperse type 1 spores is species-specific (Aylor 2017). Unlike type 1 spores, type 2 spores are released regardless of wind speed. These spores can be actively released, as with *Peronospora destructor*, which uses a hygroscopic torsion mechanism to release its sporangia (Leach 1982; Leach et al. 1982).

The architecture of the crop canopy (height, density) and the position of infection sites on plants also influence the rates of auto- and alloinfection. The higher the crop and the denser the canopy (e.g., potatoes, beans), the lower the probability that spores escape from the canopy, unless sporulation takes place on the top of the canopy. When infections are located at the base of plants instead of the top, the number of spores escaping from the canopy will also be lower. All these factors make it difficult to quantify the phases of autoinfection. It is only when a dispersion threshold corresponding to alloinfections and specific to the pathosystem is reached, that it is possible to intercept spores using spore samplers.

The understanding of spatial distribution patterns can be facilitated by a good comprehension of the structure of the plant pathogen populations. In turn, this knowledge could also help to better plan the deployment of aerobiology experiments. For example, the presence of different mating types in the same location may suggest the production of overwintering inoculum, and thus the probabilities of the inoculum being local is greater. In their study of *M. graminicola*, Zhan et al. (2002) suggests an equivalent proportion of the two sexual types at the field or country scale. In this context, the sources of inoculum may be local, and the deployment of experiments adapted accordingly. In other cases, the presence of compatible sexual types may vary from region to region. This is the case of *P. infestans* for whom the mating types A1 and A2 can be present in the same field which implies that the overwintering inoculum could play a role in the initiation of epidemics (Widmark et al. 2007). In other regions only A1 or A2 types are present, which implies an important role for external inoculum sources (Blandón-Díaz et al. 2011; Peters et al. 2014). Thus, this knowledge can also influence the planning of research initiatives (Fall et al. 2015).

3.9 Spore sampler networks

Results obtained by monitoring using spore samplers should be interpreted with great caution, as the collected spores may come from a large but distant source or from a smaller source nearby (West and Kimber 2015). For this reason, the concepts of auto- and alloinfection must also be defined at the landscape scale (region or larger territory). Thus, the plant, plot or field becomes the basic unit; inoculum accumulation locally takes place at the scale of the smallest unit in the system; and autoinfection is also defined within this unit (Mundt 2009). When a critical level is reached, spores are transported outside the boundaries of this basic unit (plant, plot or field), and it becomes possible to proceed to epidemiological surveillance. At the landscape level, it becomes possible to measure alloinfection on a small scale and auto- and allo-infection on a larger scale. It is at this scale that spore sampling networks can be deployed.

There are as many types of spore sampling networks as there are networks, in other words, there are no universal rules that apply to all networks. Of course, the structure of the network should correspond to the basic sampling objectives. Airborne inoculum monitoring is a scouting technique, so the higher the number of sensors and the frequency of sampling, the more reliable and representative the data will be. While in general it is the availability of resources that dictates how data will be collected, understanding the elements discussed in this review should enable managers, regardless of their network size, to make the best possible decisions given their own context. Ideally, one sampler would be positioned in each field of the network, but the obvious logistical constraints do not allow this kind of density. Hence, airborne inoculum monitoring networks can be built on an existing disease scouting network and structured to combine the two types of data collection, but they evolve gradually to adapt to the needs of the users (Van der Heyden et al. 2012).

Though implemented on different continents, few examples of spore sampler networks are documented in the literature. The network of passive samplers installed across the eastern United States to monitor the south-north progression of spores of Asian soybean rust (*Phakopsora pachyrhizi*) is among the most extensive. Through the ipmPIPE information platform (https://www.ipmpipe.org), this network was designed to model the progression of the disease across the US and in Canada and to demonstrate that the use of spore samplers was less costly than and as effective as sentinel plot monitoring, which had formerly been used for the network (Isard

et al. 2011). Results of this project suggest that the information collected at the continent scale can be used to limit the number of inoculum sources, but above all, to delay the progression of the disease along the south-north axis (Isard et al. 2011).

In Belgium, spore sampler networks have been assessed for monitoring the airborne inoculum of wheat yellow rust (*Puccinia striiformis f. sp. tritici*) (Duvivier et al. 2016; Hellin et al. 2018; Dedeurwaerder et al. 2011). The approach was followed in Wallonia in 2008-2009, using active volumetric samplers (7 day recording volumetric sampler). Results suggest that spore sampler networks could be used to estimate the concentration of *P. striiformis* spores and predict yellow rust epidemics (Dedeurwaerder et al. 2011). Similarly, a monitoring network including the use of spore samplers is being deployed in England to monitor various diseases, in wheat, canola and potatoes among other crops. In this example, the use of spore samplers is coupled with sentinel plots (untreated), a network of weather stations allowing the use of predictive models, and a network of collaborators providing disease observations (https://www.fera.co.uk/our-science/active-r-and-d/in-field-diagnostics). This network is operated jointly by a private research organization (Fera). The data are accessible through a web platform, and producers can subscribe to a text message alert service.

A slightly different approach is being assessed in Australia for monitoring diseases in wheat (*Pyrenophora tritici-repentis*) and canola (e.g., *Leptosphaeria maculans*). The working group is composed of researchers from the South Australian Research and Development Institute, in collaboration with the Australian Department of Agriculture and grower groups. The approach is based on the use of two types of spore samplers: fixed Cyclone and mobile Jet Spore-type mounted on the roof of a light truck. The approach is still under development (2017-2022), but a new initiative has been launched to add grapes, sugar cane, cotton, and nursery trees to the list of monitored plants.

In Brittany, the Vigispore Initiative, by the private research organization Végénov, was launched in January 2017. This initiative aims to assess the use of fixed spore samplers such as the Cyclone for monitoring airborne concentrations of *P. destructor*, *B. squamosa* and *B. allii* spores in shallot cultivation (Dr Celine Hamon, personal communication). The use of spore samplers in combination with forecast model predictions has been identified as a potential avenue to reduce the fungicide treatment frequency index in the region. This initiative, deployed on two sites in

2017 and 2018 and six sites in 2019, is inspired by the spore sampler network deployed in the Jardins-de-Napierville Regional County (Quebec) for onions.

The Quebec spore collection network mentioned above has been running annually since 2008 (Van der Heyden et al. 2012b). Unlike other networks that aim to monitor at larger spatial scales (state, country or continent), it is designed for regional monitoring at the 30-km-by-30-km scale. This spore sampler network is an example of sustainable airborne inoculum monitoring, and as such, involves researchers affiliated with private organizations, researchers from federal agencies, provincial collaborators, technical support clubs, onion producers and even, to some degree, processors. Spore sampling is carried out using rotating-arm type of samplers installed in about 20 fields, depending on the distribution of producing fields. Spore samplers are in operation three times per week and sample the air between 8h00 and 14h00 50% of the time. Spore concentrations are counted using qPCR assays and results are transmitted in near-real time to growers and their farm advisors, accompanied by the disease risks predicted by a forecasting model, so that the best decision can be made regarding appropriate phytosanitary treatments or actions (Fig. 6). The work carried out through this monitoring initiative has resulted in a significant reduction in the environmental risk index, health risk index and treatment frequency index (Carisse and Van der Heyden 2017). More precisely, this approach reduced the three indices by 32%, 14% and 28% respectively on average in 2015–2017, compared to the reference period (2007–2009).

3.10 Spore counting

The effectiveness of airborne spore monitoring systems for disease management decision making depends in particular on the speed of spore counting and the accuracy in identifying the targeted spores. The simplest method of counting in terms of equipment is certainly microscopic counting. However, in general, samples contain so many different spores and other particles that it is essential to determine what needs to be counted before starting. Although this method has been widely used, it can be long and tedious, even for experienced personnel. Several factors must be considered, including the size of the particles to be identified, the magnification and staining (aniline blue, safranin, etc.). Nevertheless, this counting method has been widely used, particularly for *B. squamosa* in onions (Carisse et al. 2012; Carisse et al. 2005; Carisse et al. 2008; Van der Heyden et al. 2012a), *P. aphanis* in strawberries (Carisse et al. 2013; Van der Heyden et al. 2014b), *P. infestans* in potatoes (Fall et al. 2015b), *B. lactucae* in lettuce (Fall et al. 2015a; Fall et al. 2016),

V. inequalis in apples (Charest et al. 2002), and many others. Visual identification and counting of spores by microscopy remain uneasy and of limited accuracy. Spores found in airborne samples generally have a different appearance than in an aqueous solution or when coming from a pure culture. They are normally dehydrated and can be deformed due to impaction and rotation of the sampling surface. In many cases, morphological characteristics of spores are very similar between species, making species identification very difficult for some spores. This is notably the case for powdery mildews (e.g., *Erysiphe necator*, *Podosphaerea aphanis*), which are relatively difficult to identify when other species of the same genus originating from nearby weeds may be present on the sample. Also, it is more and more important to distinguish genotypes (e.g., *formae speciales*, clonal lineage, races, fungicide resistant genotypes), and this is not possible on the basis of spore appearance. In addition, the presence of soil particles, dust, pollen and other debris can complicate spore identification (Fig. 7). Thus, it is often necessary to measure the dimensions of each particle and compare with reference specimens, or regularly adjust the focus to find all particles and quantify them adequately (Lacey and West 2006). Such constraints can substantially increase the time required to identify spores and complicate precise species identification. However, microscopic counts can be used to determine presence/absence or estimate categories of spore density such as low, moderate and high for a given spore species.

In order to overcome the shortcomings of microscopy in terms of specificity, standardize the counting methods and reduce the response time, different approaches have been developed over the years. Among the alternative methods, the use of monoclonal antibodies combined with a lateral flow device has been assessed for some pathogens (Kennedy and Wakeham 2008). However, these methods have very low sensitivity, and although they appear to be specific, their detection limit is often inadequate. For *Peronospora destructor*, for example, the detection limit of such a system was found to be about 500 sporangia (Kennedy and Wakeham 2008). Hence, molecular approaches using PCR and qPCR methods were assessed, and have proven to be more appropriate tools for spore counting. The first plant pathology trials were carried out in the early 2000s, with *Penicillium roqueforti* as a model organism. This trial, conducted using conventional PCR combined with agarose gel electrophoresis, allowed the specific detection of 10 spores, which represents a substantial improvement over immunoassays (Williams et al. 2001). Although it was not possible to obtain a spore concentration in the sample, this trial paved the way for several developments. Quantitative PCR methods were designed for different systems, including

Sclerotinia sclerotiorum (Rogers et al. 2009) and *Erysiphe necator* (Falacy et al. 2007). After publication of the marker for the identification and quantification of *B. squamosa* under real field conditions (Carisse et al. 2009b), the qPCR approach has been intensively used. Subsequently, molecular markers were developed for fungi including, among others, *Botrytis cinerea* (Carisse et al. 2014; Suarez et al. 2005), *Peronospora effusa* (Klosterman et al. 2014b), *P. infestans* (Fall et al. 2015c), *B. lactucae* (Kunjeti et al. 2016), *P. cubensis* and *P. humuli* (Summers et al. 2015, Rahman et al. 2020), and *Fusarium graminearum* (Hellin et al. 2018).

With air sampling, especially in agricultural fields, a variety of inhibitors may be present in the samples to be analyzed by qPCR, which can increase the frequency of false negatives. Thus, care must therefore be taken to include controls to detect these false negatives (McDevitt et al. 2007). Several approaches like the dilution of sample DNA or the use of additive and PCR enhancer such as BSA or Polyvinylpyrrolidone can be used to circumvent the presence of PCR inhibitors. A more common practice is to include a second exogenous target added to the system in a known amount that is constant from sample to sample (Haudenshield and Hartman 2011). In addition to detecting false negatives caused by the presence of inhibitors, these exogenous internal controls also enable this inhibition to be quantified and used to correct the values obtained (Bilodeau et al. 2011).

3.11 Thresholds and interpretation of aerobiological data

For an efficient disease management, it is essential to be able to predict the risk of an epidemic. Thresholds are therefore an important tool in integrated or reasoned management as they make it possible to select the best time to respond as well as the type of response. Regardless of their type, threshold are indicators that reduce the frequency of incorrect decisions (i.e., responding when unnecessary and not responding when necessary). The economic threshold is the disease severity level at which the cost of treatment becomes lower than the estimated cost of losses, whereas an action threshold, also called treatment threshold, is the disease level at which treatments is less effective due to high disease pressure. A damage threshold or tolerance threshold is reached when the estimated losses at harvest are higher than the acceptable disease level (for exemple: 5% grey mould severity in grapes).

Thresholds are established in accordance with the relationship between the values of the indicator (disease incidence or severity, spore concentration) and the damage (loss of yield, lower quality) (Fig. 3.8). When this relationship is known, as well as the acceptable damage level, it is

simple to establish the threshold. However, in practice, the threshold varies with the crop development stage, the expected harvest date, the type of market (fresh, storage, processing), and the grower's risk tolerance level. Fungal diseases with aerial dispersion are generally referred to as "polycyclic," i.e., the population of the plant pathogenic fungus increases with each secondary reproductive cycle. The number of reproductive cycles is difficult to predict because it depends on the fungus, the crop's sensitivity, weather conditions, and the length of the growing season.

Action thresholds based on airborne spore concentration are difficult to define, as the concentration varies with the number and proximity of spore sources. For this reason, information on spore concentration is often interpreted in terms of the time of capture of the first spores (Thiessen et al. 2016; Van der Heyden et al. 2012a) and the increase in the aerial spore concentration (Carisse et al. 2012). For onion leaf blight (*Botrytis squamosa*), a first action threshold was developed by comparing airborne concentration of conidia with number of lesions per leaf under field conditions. The threshold was determined based on concentrations corresponding to disease-based thresholds already used to time fungicide applications with values of 10-15 and 25-35 conidia per m^3 corresponding to 1 and 2.5 lesions per leaf, respectively (Carisse et al., 2005) (Fig. 3.9a). Based on this study, airborne inoculum-based thresholds (first conidia and total of 15 conidia per m^3 caught) were compared to a disease-based threshold (one lesion/leaf) to time the initiation of fungicide spray program for onion leaf blight management (Van der Heyden et al. 2012) (Fig. 3.9b). In this study, best disease management was obtained when fungicide spray programs were initiated at first airborne *B. squamosa* conidia detected, followed by a threshold of a total 15 conidia per m^3, and by initiation when the first lesion was detected. To further improve onion leaf blight management, a disease risk indicator was developed by combining weather-based risk of infection, amount of airborne inoculum and forecasted favorability of weather conditions for infection (Carisse et al. 2012).

Regardless of the approach followed, thresholds are highly dependent on the sampling method and disease management strategy. When the conditions of use of the spore samplers are well-defined and validated according to the development of diseases, the threshold must then be determined. In other words, the question is how to express the number of captured spores into risk and the decision of applying or not a fungicide treatment (synthetic or biological). Each fungus has its own survival strategy. Some produce a large number of spores when one individual spore has little chance of infecting a plant. In this case, the threshold will be high, e.g., with powdery

mildews (strawberries, grapes). For example, Van der Heyden et al (2014b) find a linear relationship between airborne concentration of *Podosphaera aphanis* conidia with strawberry powdery mildew severity and reported that 5 and 10% disease severity corresponded to 50 and 100 conidia/m3. Other fungi produce fewer, very aggressive spores, as is the case with downy mildews (potatoes, vines). In these cases, thresholds must be lower. Thresholds should be determined based on controlled studies and field observations. Fall et al. (2015) studied the relationship between airborne sporangia concentration of four clonal lineages of *Phytophthora infestans* (US-8, US-11, US-23, and US-24) and late blight of potato development under controlled conditions. Even though the shape of the disease-inoculum curve were similar for all clonal lineages, infection efficiency of sporangia was different. The sporangia concentration required to cause one lesion per leaf was 10 sporangia per m^3 for US-23 and 15 to 25 sporangia per m^3 for the other clonal lineages of *P. infestans*.

Data from spore samplers should be interpreted by considering many other risk factors such as weather, crop susceptibility, disease level, and aggressiveness of the pathogen (Carisse et al. 2012; Fall et al. 2015b). A large number of spores in conditions that are unfavorable to disease development may represent a lower risk than a small number of spores under favorable conditions. Thresholds should also be interpreted in accordance with the growth stage of the crop. Therefore, the presence of spores does not necessarily mean that an action (treatment) is necessary.

3.12 Perspectives

In recent decades, global trade in plant materials and products has contributed to the spread of many pathogens. In addition, climate change tends to increase the length of the growing season, especially in northern territories (Evans et al. 2008). Consequently, these changes could influence seasonal epidemics of resident (endogenous) pathogens, promote the production and survival of overwintering inoculum, increase the frequency and scale of seasonal epidemics for exogenous pathogens, and change the status of some plant pathogens from periodically introduced to endemic (Van der Heyden et al. 2020). Moreover, pesticides use is under heavy scrutiny, and growers feel the pressure of the public, buyers and consumers, to reduce their dependence on synthetic pesticides. Tools are needed for the industry to estimate short- and middle-term risks and to identify the most appropriate control measures and times to apply phytosanitary measures, including those with low environmental risk. For governments, indicators to monitor the impacts

of climate change and introduced pathogens are required. Monitoring the territory, particularly with networks of spore samplers, is perfectly coherent in this context.

The development of quantitative molecular approaches has allowed the deployment of spore sampler networks at different scales (Carisse et al. 2012; Carisse and Van der Heyden 2017; Fall et al. 2015c; Van der Heyden et al. 2012a, Chen et al. 2018). The combination of these approaches allows an accurate monitoring of a predetermined number of pathogens, usually between one and four. However, molecular methods are evolving rapidly, and new promising approaches are continuously being developed. As an example, scientists are currently attempting to combine isothermal PCR technologies (LAMP-PCR, RPA-PCR) and automated spore sampling instruments to send real-time risk alerts to growers and crop specialists (West et al. 2018). Presently, these devices are expensive (~$30,000 USD per unit), their availability is limited, and the number of pathogens that they can monitor simultaneously is also limited. Several research groups are working on the development of molecular tools compatible with these approaches to combined spore sampling and real-time data transmission (Lees et al. 2019; Si Ammour et al. 2017). Automated image recognition technologies are also progressing rapidly, and with them, a true real-time monitoring approach is getting closer (see https://www.scanittech.com or https://bioscout.com.au).

It is impossible to ignore the emerging technology of single-molecule sequencing, also known as third-generation sequencing, and its potential to improve biomonitoring at multiple scales. This technology could quickly become a benchmark in terms of diagnosis and has several advantages over previous approaches. First, it allows the sequencing of long DNA fragments that are easier to assemble and align with reference genomes and sequencing entire small and medium-sized genomes such as those of bacteria and certain fungi. Presently, there are two third-generation sequencing platforms: Pacific Biosystems (PacBio) and Oxford Nanopore Technologies (ONT). The ONT platform offers a faster response time (between 3 and 5 hours), requires only a small amount of initial DNA, and has lower costs of acquisition (Hu et al. 2019). In 2014, ONT launched the first portable third-generation sequencer, the MinION. It's small size and ability to connect to a local computer make it suitable for use in all laboratories. Several bioinformatics tools have been adapted for the MinION sequencer and allow real-time identification of plant pathogenic species present in a sample. This new technology has already been shown to be useful for phytosanitary biomonitoring (Chalupowicz et al. 2018; Bronzato Badial et al. 2018). In these trials, a number of

plant species (e.g., tomato, pepper, melon, Gypsophila, strawberry, lemon) presenting unknown symptoms or knowingly inoculated with 16 bacterial, fungal and viral pathogens, were analyzed using Nanopore's tools. For all inoculated or symptomatic plants, the pathogen species were identified within 2 hours (Chalupowicz et al. 2018).

Since the discovery that some pathogens are dispersed in the air, aerobiology has evolved from the establishment of principles (Gregory 1945) to molecular identification and quantification of airborne spores (Carisse et al. 2009), and statistical and mathematical modeling (Aylor 2017). Nowadays, the combined use of high throughput sequencing (HTS) and bioinformatics in aerobiology, allows the simultaneous identification of several targets. An example of this new approach is presented in Chen et al. (2018) were the use of metagenomics using HTS is combined to a spore-sampling network (AeroNet) to detect specific taxonomic groups. In an example from forestry in Canada, Tremblay et al. 2018 identified regulated and unregulated pathogenic forest fungi in spore trap samplers. They identified potential species to be controlled as they could cause diseases not yet recorded in the region (Berubé et al. 2018a, b) and may require management procedures. In Europe, HTS methods have been used to study fungal diversity in different environmental substrates. In this study, it was shown that the assessment of airborne fungal community diversity can be achieved by using simple and robust passive spore traps (cellulose filters, coated microscope slides) (Aguayo et al. 2018). Redondo et al (2020) evaluated the airborne fungal spore community and the fungal spore community deposited with active and passive spore traps in three different vegetation types in Sweden using metagenomics.

The use of these new technologies also accelerates the discovery of new genotypes with specific epidemiological characteristics, in terms of aggressiveness, host specificity or resistance to fungicides. Combined with the use of spore samplers organized in networks, these new tools will make it possible to carry out genotype-specific biosurveillance. Examples of this type of monitoring are not yet very abundant, but it is likely that this type of use will become more and more common. Among the available examples is the development of a monitoring tool for two host specialized clades of *P. cubensis* (Rahman et al. 2020), identified as a result of a genome wide association study (Wallace et al. 2020). Similarly, tools for simultaneous monitoring of *Plasmopara viticola* clade *riparia* and clade *aestivalis* was recently developed (Carisse et al., 2020). Another example of such an approach is provided for *Z. tritici*, for which molecular markers have been developed to monitor specific DNA substitutions associated with fungicide resistance

to demethylation inhibitors and succinate dehydrogenase (Hellin et al. 2020). Hence, it is expected that aerobiology will continue to evolve through the integration of new technologies such as metagenomics, but also through other knowledge such as deep learning, neural networks or more automated methods of sample analysis.

In conclusion, airborne inoculum monitoring networks combined with advanced DNA analysis, image and data analysis technologies open the door to a new generation of crop disease management tools and to better understanding of pathosystems. Regardless of the level of sophistication, once in place, airborne inoculum monitoring networks allow early detection of resident or immigrant pathogens; thus, control measures can be deployed before the disease gets out of control. In addition, DNA analysis can detect changes in the population of plant pathogens such as virulence or genetic substitutions related to resistance to fungicides. The observations from the networks allow the identification of the source (reservoir) of inoculum, to describe the dispersion of the pathogen at a territory scale, to identify the zones most at risk (hotspots), and to study the impact of climate change and cultural practices on the development of diseases. In other words, to improve our knowledge of pathosystems. As research data and data from inoculum tracking networks become available, it will be possible to specify the best location and improve spatial resolution.

In the context of the 'big data' era, airborne inoculum monitoring networks are still in the juvenile phase and research must be done essentially to integrate available technologies, improve access, analysis, interpretation and data sharing. It is likely that with our constant need for more and more data, that local networks will be integrated into regional networks and then into national networks. It is therefore essential to properly structure the root networks; it is in this spirit that we have prepared this literature review.

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Figure 3-1. Examples of spore samplers.A) Passive sampler comprising two microscope slides mounted horizontally on a wooden pole; B) rotating-arm impactors; C) Burkard 7-day recording volumetric sampler; D) funnel-like passive spore sampler from Spornado (courtesy of Kristine White and Yaima Arocha Rosete, Sporometrics); and E) Burkard high-volume cyclone (courtesy of Celine Hamon, Vegenov).

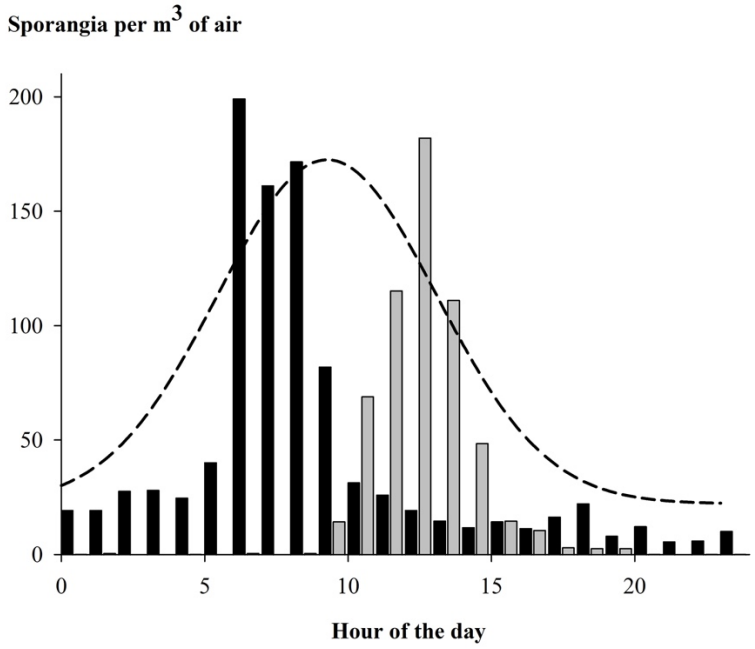


Figure 3-2. Example of periodicity for *Bremia lactucae*.Periodicity can be defined as the description of circadian cycles of spore emission. Emission peaks are influenced by relative air humidity, drying of foliage, and wind speed. Splitting sampling over time extends the sampling time and therefore makes the sampling more representative. The grey and black bars represent two distinct sampling days.

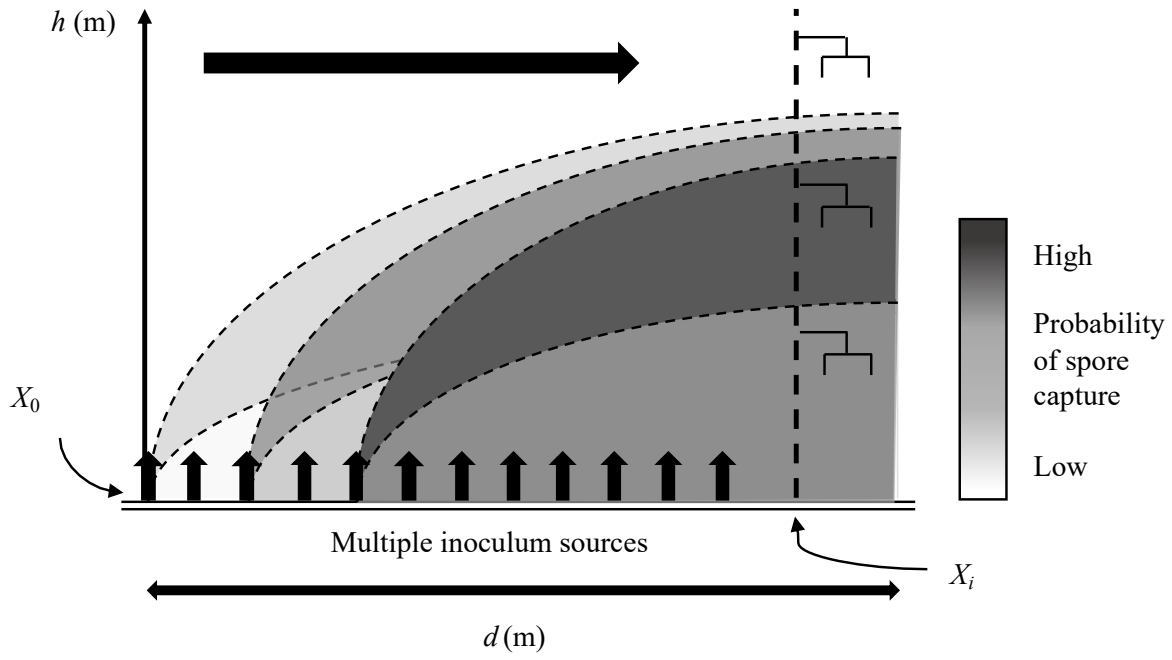


Figure 3-3. Horizontal flows of spores in the direction of the wind correspond to the cumulative concentration of spores escaping from the canopy (for each source) between the edge of the field (or first source) and the location of the sampler. Spore concentrations become almost constant at distance *d* from the point X_0 at the edge of the field (or plot) in the direction of the wind from the sampler location (X_i). Adapted from Aylor (2017).

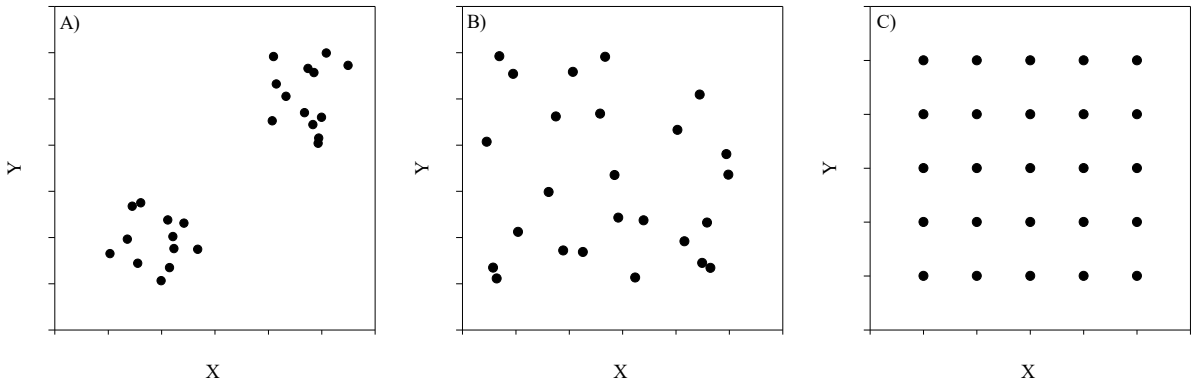


Figure 3-4. Main types of 2D spatial point patterns. A) Aggregated: diseased plants are grouped in foci. B) Random: areas of same size in the field have equal probabilities of containing the same number of diseased plants. C) Regular: diseased plants are distributed at regular intervals.

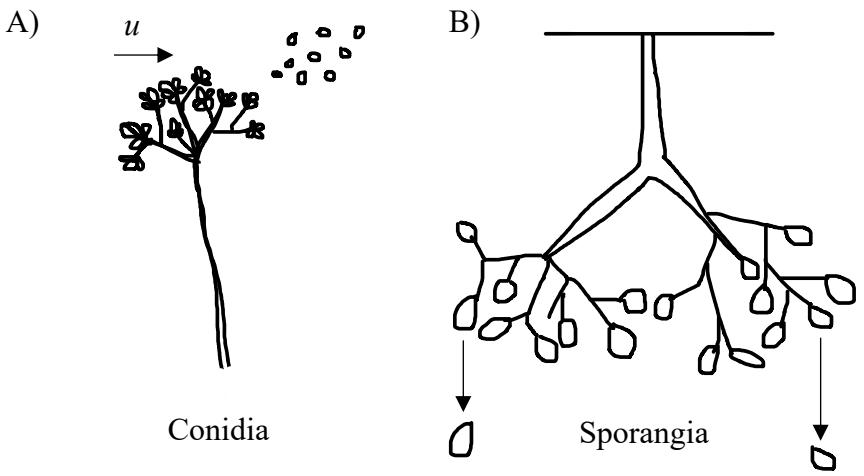
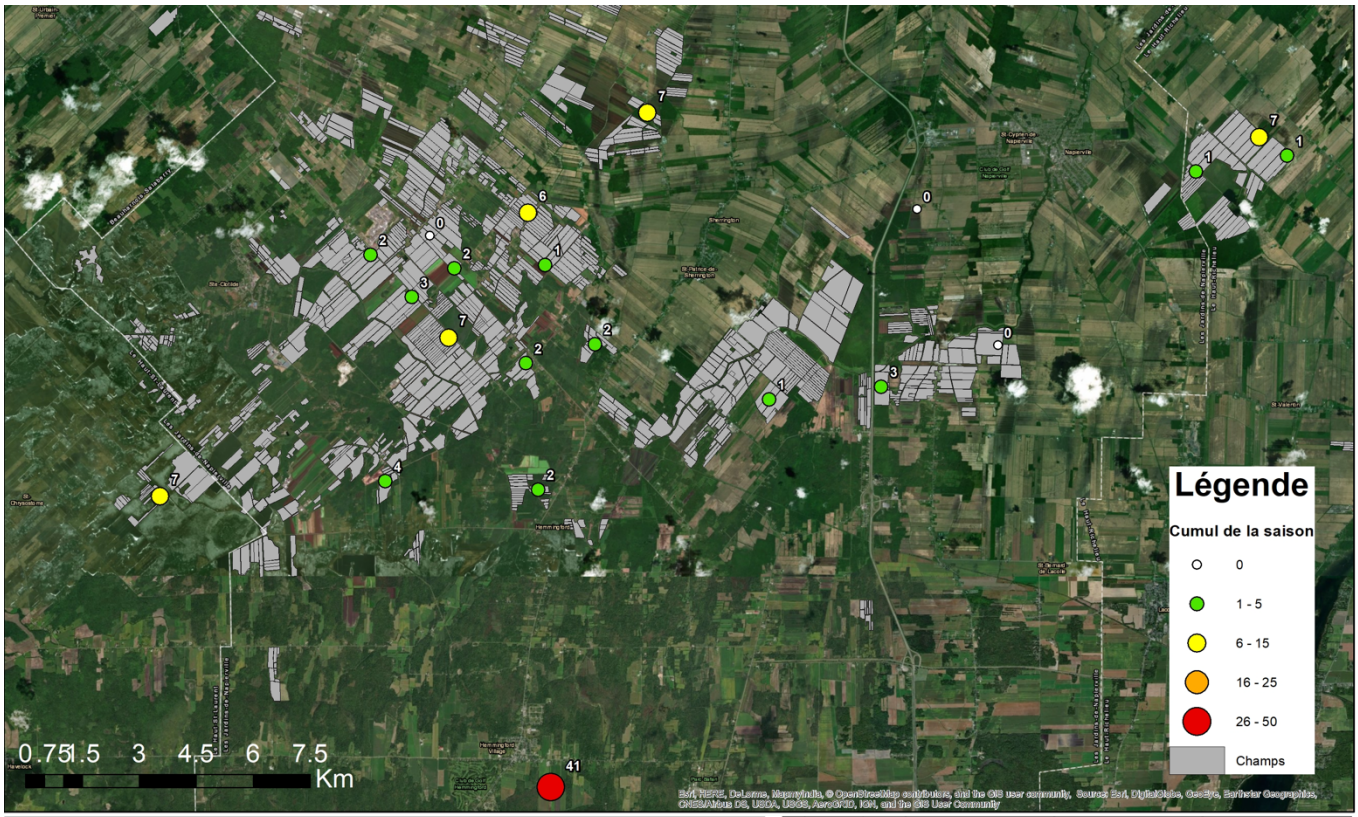


Figure 3-5. Types of spores according to their release mechanism: A) passive release (type 1) and B) active release (type 2). Adapted from Aylor (2017).



No	Producteur	Champ	24-Jun-18			26-Jun-18			28-Jun-18		
			Botrytis	Mildiou	Stemphilium	Botrytis	Mildiou	Stemphilium	Botrytis	Mildiou	Stemphilium
1	DELF	E6	.	.	.	0	0	0	1	0	0
2	DELF	30	.	.	.	0	0	0	0	0	0
3	DELF	70	.	.	.	0	0	0	0	0	0
4	VERN	87	0	0	0	0	1	0	0	0	0
5	VANH	G5	0	0	0	0	3	0	0	0	0
6	VERN	100	0	0	0	0	0	0	0	0	0
7	VANW	52	0	2	0	0	0	0	1	3	1
8	BARR	B4	0	0	0	0	0	0	0	0	0
9	SOREL	64	0	0	0	0	0	0	0	0	2
10	LEGM	30	0	0	0	.	.	.	0	0	0
11	LEFO	L3	0	0	0	.	.	.	2	1	2
12	LEGM	St-Remi	0	.	.	0	0	0	0	0	0
13	COUS	CL14	0	0	0	0	0	0	0	0	0
14	DIEC	72	0	0	0	0	0	0	2	0	2
15	GUEA	2P	0	0	0	0	2	0	0	0	0
16	GUEF	R55-56	0	0	0	1	0	0		0	0
17	ISAB	S1	0	0	0	0	0	0	0	0	0
18	JSTC	3	0	0	0	.	.	.	0	0	0
19	GUEJ	1.3	2	2	0	0	0	0	0	0	0
20	LECL	106	0	0	0	0	0	0	1	0	0
21	REMR	GP7	0	0	0	0	0	0	1	0	0
22	ZUMS	NB	0	2	0	0	0	0	0	1	0
23	LAMO	-	.	.	.	0	0	0	0	2	0

Figure 3-6. Example of results distributed to onion producers and their farm advisors, from the spore sampler network established in southwestern Québec in 2008.

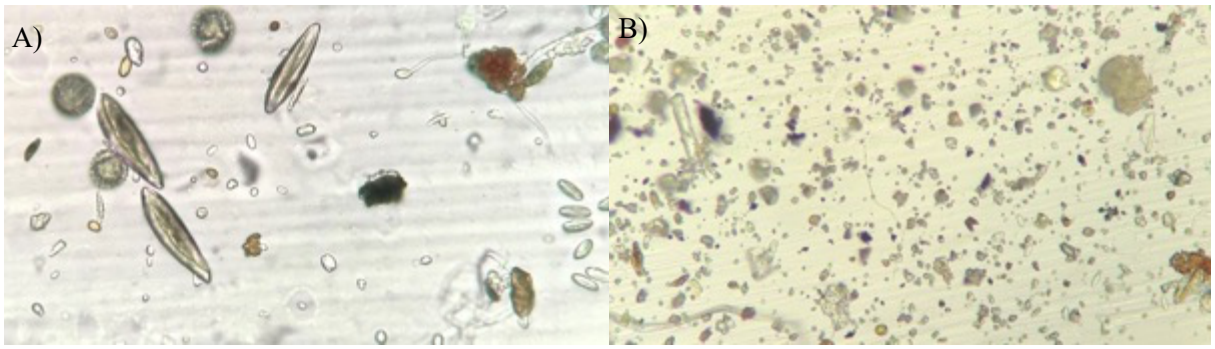


Figure 3-7. Examples of airborne samples collected using rotating-arm impaction sampler, showing A) Peronospora destructor sporangia with few debris and B) a sample containing a greater amount of debris.

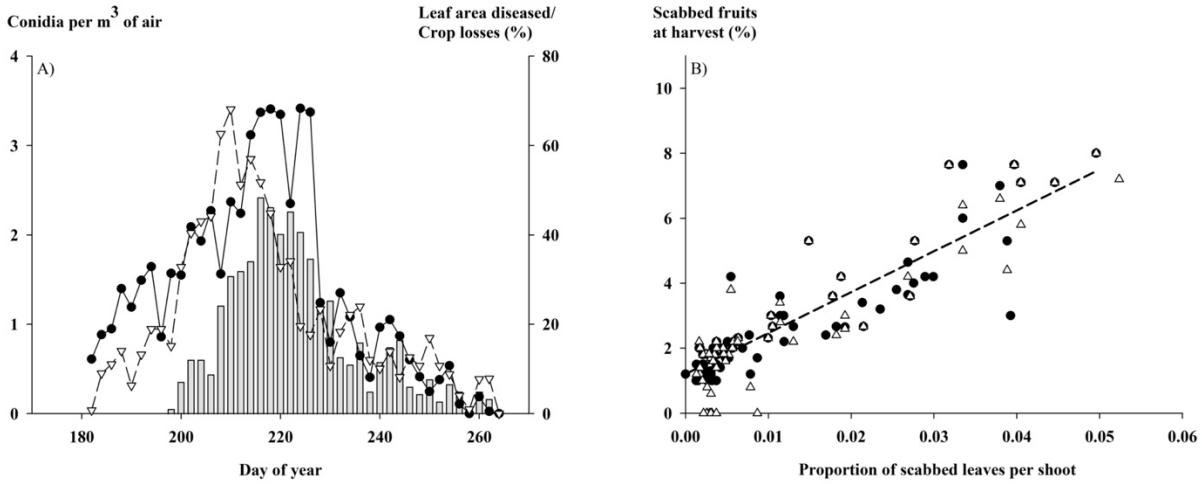


Figure 3-8. Thresholds established in accordance with the relationship between the values of the indicator and the damage. (A) Plots of the severity of powdery mildew on strawberry leaves (open down-pointing triangles), the airborne concentration of *Podosphaera aphanis* spores (black circles) and crop losses (grey bars) in relation to the day of year. (B) Proportion of scabbed leaves per shoot (*Venturia inaequalis*) at the beginning of the growing season and the percentage of scabbed fruit at harvest. The black circles were used for model development and empty triangles for validation purposes (Carisse et al. 2009; Carisse et al. 2012; Carisse et al. 2013).

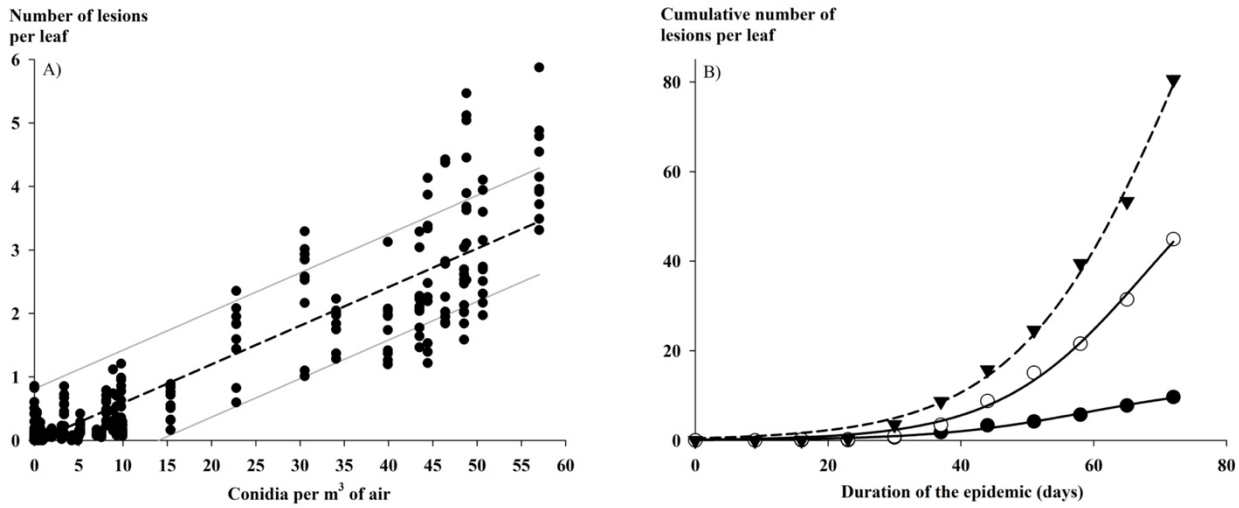


Figure 3-9. Action thresholds based on airborne spore concentration. A) Relationship between an economic treatment threshold and the aerial concentration of *Botrytis squamosa* spores. The dotted black line is the linear regression, and the solid grey lines represent the prediction interval (Carisse et al. 2005). B) Relationship between the progression of onion leaf blight and the indicator used to initiate the treatment program; down-pointing black triangles correspond to the threshold of 1 lesion per leaf, open circles to the threshold of 15 spores per m^3 of air, and black circles to the threshold of 1 spore per m^3 of air (van der Heyden et al. 2012a).

Table 3-1. Published studies and aerobiological datasets in relation to purposes and specific objectives.

Purpose	Specific objective	Pathogen	Disease	Crop	Type of spore sampler	Scale	Sampling height (m)	Quantification method	Reference
Decision support system	Use ASC to predict disease secondary infection in vineyards	*Plasmopara viticola*	Downy mildew	Grape	7-day recording volumetric	Plot	1.5	Microscopy	(Brischetto et al. 2020)
	Development of predictive models based on ASC[1] and weather	*Blumeria graminis* f. sp *tritici*	Powdery mildew	Wheat	7-day recording volumetric	Field	0.6	Microscopy	(Cao et al. 2014)
	Establish thresholds of airborne conidial concentrations	*Botrytis squamosa*	Botrytis leaf blight	Onion	Rotating-arm impactor	Field	0.15 (above canopy)	Microscopy	(Carisse et al. 2005)
	Characterize the relationship between ASC and disease incidence, and test action thresholds	*Erysiphe necator*	Powdery mildew	Grape	Rotating-arm impactor and 7-day recording volumetric	Field	0.4, 0.45 and 1.5	Microscopy	(Carisse et al. 2009)
	Development of a risk indicator-based ASC	*B. squamosa*	Botrytis leaf blight	Onion	Rotating-arm impactor	Field	0.15 (above canopy)	Microscopy	(Carisse et al. 2012)
	Development of a risk indicator-based ASC	*Bremia lactucae*	Downy mildew	Lettuce	Rotating-arm impactor	Field	0.5	qPCR (probe)	(Dhar et al. 2019)
	Development of a qPCR assay to study the distribution of ASC and disease epidemics	*Puccinia triticina*	Leaf rust	Wheat	7-day recording volumetric	Region	1	qPCR (probe)	(Duvivier et al. 2016)
	Detecting risk periods of development and infection to reduce fungicide treatments	*Botrytis cinerea*	Grey mold	Grape	7-day recording volumetric	Field	2	Microscopy	(Fernando et al. 2000)
	Development and implementation of a LAMP-based assay for detection of ASC	*E. necator*	Powdery mildew	Grape	Rotating-arm impactor	Plot	Above canopy	qPCR - Lamp PCR	(Thiessen et al. 2016)
	Comparison of action thresholds for initiation of a fungicide spray program	*B. squamosa*	Botrytis leaf blight	Onion	Rotating-arm impactor	Region/Network	0.15 (above canopy)	Microscopy	(Van der Heyden et al. 2012)
	Development of a monitoring procedure to study ASC temporal distribution and level of association with disease	*Fusarium graminearum*	Fusarium head blight	Wheat	7-day recording volumetric	Contry/Network	1	qPCR (probe)	(Hellin et al. 2018)

	Development of a detection tool for in-field ASC monitoring and integration into existing weather-based forecasting systems	*Phytophthora infestans, Alternaria solani*	Late and early blight	Potato	Rotating-arm impactor	Field	NA[2]	qPCR (probe) and LAMP	(Lees et al. 2019)
	Development and validation of qPCR assay based on species- and clade-specific markers for biosurveillance	*Pseudoperonospora cubensis*	Downy mildew	Cucumber	Rotating-arm impactor	Plot	1	qPCR (probe)	(Rahman et al. 2020)
	Development of a qPCR-based monitoring approach for disease forecasting	*Sclerotinia sclerotiorum*	Stem rot	Oilseed rape	7-day recording volumetric	Field	0.7	qPCR (probe)	(Rogers et al. 2009)
	Development of a qPCR-based assay for detection of ASC	*Peronospora humuli*	Downy mildew	Hop	Rotating-arm impactor	Field	1.5	qPCR (probe)	(Summers et al. 2015)
	Assess possible reduction of the use of sentinel plots by spore trapping and modeling	*Phakospora pachyrhizi*	Soybean rust	Soybean	Rain collector	Country/network	N/A	qPCR (probe)	(Isard et al. 2011)
Analysis of spore dispersal	Study spore flux in relation with wind and estimate spore escape from a diseased field	*Peronospora tabacina*	Blue mold	Tobacco	Rotating-arm impactor	Plot	0.75-4.0	Microscopy	(Aylor and Taylor 1983)
	Measurement of ASC at various heights above the ground in apple trees	*Venturia inaequalis*	Scab	Apple	Rotating-arm impactor	Plot	0.15-3.0	Microscopy	(Aylor 1995)
	Development of a model-based approach for quantifying atmospheric dispersal	*P. infestans*	Late blight	Potato	Rotating-arm impactor and 7-day recording volumetric	Plot	0.71 - 2.81	Microscopy	(Aylor et al. 2001)
	Study spore dispersal in relation with wind and correlate disease with ASC	*Alternaria alternata*	Leaf blight	Cotton	Passive (petri dish)	Field	1.5	Microscopy	(Bashan et al. 1991)
	Validation of a dispersion model to estimate source strength and deposition	*Puccinia triticina*	Rust	Wheat	Rotating-arm impactor	Plot	0.5, 1.0 and 2.0	Microscopy	(Chamecki et al. 2011)
	Investigate the role of rain and wind in the dispersal of ASC	*Botrytis fabae*	Chocolate spot	Beans	Passive trap and 7-day recording volumetric	Plot	0.4	Microscopy	(Fitt et al. 1985)
	Study the pattern of spore dispersal in relation to crop biology, disease incidence and weather variables	*Botrytis cinerea*	Grey mold	Raspberry	7-day recording volumetric	Field	1	Microscopy	(Jarvis 1962)

	Improve the understanding of dispersal patterns for genes, genotypes and populations	*Blumeria graminis* f. sp *hordei*	Powdery mildew	Barley	Volumetric jet spore	Country	25	Colony count	(Limpert et al. 1999)
	Study temporal dynamics, escape from the canopy, and relationship between ASC and disease development	*Pseudoperonospora cubensis*	Downy mildew	Cucumber	Rotating-arm impactor	Plot	0.5-3.0	Microscopy	(Neufeld et al. 2013)
	Understand ASC over the winter months and long-distance dispersal	*Peronospora effusa*	Downy mildew	Spinach	Rotating-arm impactor	Region	NA	qPCR (probe)	(Choudhury et al. 2016)
	Study disease spread from a small area inoculum source	*F. graminearum*	Fusarium head blight	Wheat	7-day recording volumetric	Plot	0.7	Microscopy	(Fernando et al. 1997)
Determination of the role of inoculum in disease development	Estimate the number of infections likely triggered by exposure to known concentrations of ASC	*V. inaequalis*	Scab	Apple	7-day recording volumetric and rotating-arm impactor	Plot	0.15-1.6	Microscopy	(Aylor and Kiyomoto 1993)
	Study the relationships between disease incidence, environmental conditions and ASC	*B. cinerea*	Grey mould	Strawberry	7-day recording volumetric	Plot	0.4	Microscopy	(Blanco et al. 2006)
	Study the dynamics and relationships between ASC, environmental conditions and disease index	*B. graminis* f. sp *tritici*	Powdery mildew	Wheat	7-day recording volumetric	Field	0.6 and 1.6	Microscopy	(Cao et al. 2012)
	Study the influence of disease severity, ASC and weather variables	*Podosphaera aphanis*	Powdery mildew	Strawberry	Rotating-arm impactor	Field	0.15 (above canopy)	Microscopy	(Carisse et al. 2013)
	Improve the understanding of epidemics	*B. cinerea*	Grey mould	Raspberry, strawberry and grape	Rotating-arm impactor	Field	0.35, 0.9, 1.0	qPCR (probe)	(Carisse et al. 2014)
	Study the influence of environmental factors on infection efficiency	*B. lactucae*	Downy mildew	Lettuce	Rotating-arm impactor	Plot	0.7	Microscopy	(Fall et al. 2015a)
	Connect ASC to different types of agricultural lands	*Alternaria* spp.	N/A	N/A	7-day recording volumetric	Region	16	Microscopy	(Fernández-Rodríguez et al. 2015)
	Assess the influence of ASC on the precocity of disease development	*Zymoseptoria tritici*	Septoria blotch	Wheat	7-day recording volumetric	Plot	0.7	qPCR (probe)	(Morais et al. 2016)
Determination of population diversity	Development of a qPCR assay to monitor mutations associated with fungicide resistance	*Mycosphaerella graminicola*	Leaf blotch	Wheat	7-day recording volumetric	Plot	1	qPCR (probe)	(Fraaije et al. 2005)

	Develop a qPCR assay allowing for the detection of mutation related to fungicide resistance from air samples	*Zymoseptoria tritici*	Septoria blotch	Wheat	7-day recording volumetric	Field	0.7	qPCR (probe)	(Hellin et al. 2020)
	Evaluation of passive samplers to describe airborne fungal communities by metabarcoding analysis	Fungal communities	N/A	N/A	Passive samplers	N/A	1	HTS[3]	(Aguayo et al. 2018)
	Set temporal and spatial baselines for airborne disease detection following a metabarcoding approach and evaluate the performance of three types of spore samplers in detecting taxonomic groups	Fungal communities	N/A	N/A	Cyclone and wet deposition sampler	Country	1.2	Pyrosequencing	(Chen et al. 2018)
	Study the level of fungal diversity, the proportion of phytopathogenic fungi and factors of variation in airborne communities	Fungal communities	N/A	N/A	7-day recording volumetric	Regional	1	qPCR and HTS	(Nicolaisen et al. 2017)
	Investigate the feasibility of air sampling for studying patterns of fungal diversity	Fungal communities	NA	NA	Cyclone	Continental	Ground level	NGS[4]	(Ovaskainen et al. 2020)
	Determine the effect of genotype unit area on the effectiveness of disease reduction in genetically diverse host populations	*Blumeria graminis* f. sp. *avenae*	Powdery mildew	Oat	Rotating-arm impactor	Plot	0.15 (above canopy)	Microscopy	(Mundt and Browning 1985)
Characterisation of spatiotemporal patterns	Study the spatiotemporal patterns of disease and ASC, and establish the degree of their spatiotemporal association	*B. squamosa*	Botrytis leaf blight	Onion	Rotating-arm impactor	Field	0.15 (above canopy)	Microscopy	(Carisse et al. 2008)
	Describe the spatial distribution patterns of primary inoculum and correlate them with disease	*V. inaequalis*	Scab	Apple	Rotating-arm impactor	Field	0.4	Microscopy	(Charest et al. 2002)
	Diurnal periodicity of airborne sporangia	*P. cubensis*	Downy mildew	Cucumber	Kramer-Collins	Plot	N/A	Microscopy	(Cohen and Rotem 1971)
	Study the influence of meteorological parameters on the spatial and temporal dynamics of the CSA, and explore its value as a decision support system.	*P. infestans*	Late blight	Potato	Rotating-arm impactor	Region	2.9	Microscopy	(Fall et al. 2015b)

Develop a 2D spatial model for disease foci	*F. graminearum*	Fusarium head blight	Wheat	7-day recording volumetric	Plot	0.7	Microscopy	(Paulitz et al. 1999)
Quantify temporal changes of ASC and determine influence of environmental conditions on spore release	*B. cinerea* and *S. sclerotiorum*	Blossom blight	Alfalfa	Cyclone	Field	1	qPCR (probe)	(Reich et al. 2016)
Study the relationship between ASC and disease development, the spatiotemporal structure of epidemics and the reliability of spore samplers for assessing ASC under commercial conditions	*P. aphanis*	Powdery mildew	Strawberry	Rotating-arm impactor	Field	0.5 and 1.5	Microscopy	(Van der Heyden et al. 2014)

[1] ASC stand for airborne spore concentration.

[2] N\A is for not applicable.

[3] HTS is for high throughput sequencing.

[4] NGS is for Next generation sequencing.

Table 3-2. Description of the main types of volumetric spore samplers (adapted from West and Kimber, 2015).

Category	Sampler[1]	Flow rate (L/min.)	Efficiency d50 (μm)[2]	Collection surface	Sampling period	Reference
Impaction	Burkard 7 Day	10	2.2	Tape	7 days, hourly	(Lacey and West 2006)
	Andersen, Marple Series 290, Air Trace Environmental	2-28.6	0.43-21.3	Culture medium on Petri dish	2-20 min	www.newstarenvironmental.com www.pmeasuring.com (West and Kimber 2015)
	Rotorod	100-150	>10	Sticks	Typically, 2-4 h	(Lacey and West 2006)
	Air-O-Cell (or equivalent)	4-15	>1	Slide	5-15 min	www.zefon.com
Cyclone	Burkard cyclone	16-20	>20	1.5 mL tube	24 h	(Bock and Cotty 2006)
	Coriolis μ (or equivalent)	10-630	1	20 mL tube	1-10 min	(Carvalho et al. 2008)
	CIP10-M	10	1.8	N/A	1-200 min	(Nieguitsila et al. 2011)
Electrostatic	Ionic spore trap	660	>2	Electrode	48 h	www.ionicsporetrap.com
Virtual impaction	Burkard Jet Spore Sampler	850	>2	N/A	24 h and more	(Limpert et al. 1999)
	Miniature virtual impactor	20	>2	N/A	24 h and more	(West and Kimber 2015)

[1] The main volumetric spore samplers are described in this table. There are other samplers; see West and Kimber (2015) for a more exhaustive list.

[2] d50 corresponds to the spore diameter beyond which >50% of the particles are collected and below which <50% are collected.

Connecting text for chapter 4

In southwestern Quebec, agronomists and crop specialists reported that the frequency of onion downy mildew outbreaks has increased over the years. In Chapter 4, a long-term dataset of scouting observations collected over 31 years (1987-2017) was used to provide evidence that climate change contributes to an increase in the frequency of onion downy mildew epidemics, along with epidemics onset. To determine which environmental factors were associated with these changes in epidemics, I created variables related to the different phases of the life cycles of the pathogen (sporulation, infection, production of resting structures, and survival). Then, using principal component and discriminant analyses, I evaluated which phases of the *P. destructor* life cycle were most influenced by climate change.

This chapter was submitted and is now published in a special issue of Agronomy entitled "Plant Disease Epidemiology: Changing Perspectives, Emerging Technologies, and Prediction Modeling". This special issue focuses on how epidemiology can improve our understanding of crop disease development in a changing environment, predict future disease management needs, and design new approaches to plant disease management. This manuscript is now published as:

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4 Factors Influencing the Occurrence of Onion Downy Mildew (Peronospora destructor) Epidemics: Trends from 31 Years of Observational Data.

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4.1 Abstract

Onion downy mildew (ODM) caused by *Peronospora destructor* has been increasing annually in south-western Québec since the early 2000s, reaching 33% of affected onion fields in 2014. Using observational data collected over a period of 31 consecutive years, this study aimed to investigate the variations in ODM incidence and epidemic onset and identify the meteorological variables that influence its polyetic development. A logistic model was fitted to each ODM epidemic to estimate and compare the onset of epidemics on a regional basis. Results of this analysis showed that the first observation date, 10% epidemic onset (b_{10}) and mid-time (b) were, on average, 30.4, 15.1 and 11.3 days earlier in 2007–2017 than in 1987–1996. Results of a principal component analysis suggested that regional disease incidence was mostly influenced by the precipitation regime, the final regional disease incidence the previous year, and warmer temperature during the harvest period the previous fall. Subsequently, the data were divided in three periods of 10, 10 and 11 years, and a discriminant analysis was performed to classify each year in the correct period. Using a sufficient subset of five discriminating variables (temperature and rainfall at harvest the previous fall, winter coldness, solar radiation, and disease incidence the previous year), it was possible to classify 93.5% of the ODM epidemics in the period where they belong. These results suggest that *P. destructor* may overwinter under northern latitudes and help to highlight the need for more research on overwintering and for the development of molecular-based tools enabling the monitoring of initial and secondary inoculum.

4.2 Introduction

Over the last 20 years, the world production of onion (*Allium cepa* L.) more than doubled, moving from 2.4 million hectares of land in 1997 to 6.3 million hectares in 2017 (FAO 2017). In Canada, the provinces of Ontario and Québec grow more than 90% of the national production, with 2460 ha and 1938 ha, respectively, dedicated to the production of dry bulb onions (Mailvaganam 2017). In Canada and elsewhere, onion crops are threatened by several diseases, including Botrytis leaf blight, Stemphylium leaf blight, purple blotch and downy mildew.

Onion downy mildew (ODM), caused by the obligate biotrophic oomycete *Peronospora destructor* (Berk.) Caspary, is a widespread disease that is often difficult to manage and can be destructive, with yield losses reaching up to 75% (Araújo et al. 2017; Develash and Sugha 1997; O Brien 1992; Gerald and Obrien 1994). Weather conditions favoring *P. destructor* sporulation and infection were thoroughly studied in the 1980s, following severe disease outbreaks that occurred between 1977 and 1980 in Ontario (Canada) and in western and central New York (Hildebrand and Sutton 1982; Smith et al. 1985). The *P. destructor* sporulation process is dependent on daily cycles of light and darkness and is not initiated when infected onions are kept in continuous light or darkness (Yarwood 1937). Relative humidity greater than 95% and temperatures between 4 °C and 24 °C are required during the night cycle for sporulation (Hildebrand and Sutton 1982). When humidity is high from 20:00 onwards, sporulation may occur at temperatures as low as 10 °C, whereas the optimal temperature rises to 18 °C when humidity onset is after 3:00 (Hildebrand and Sutton 1984b). After infection, a latency period of 13 days is observed at warmer temperature (25 °C/17 °C for day/night) and 15 to 17 days at cooler temperature (18 °C/10 °C for day/night) (Hildebrand and Sutton 1984c). Mature sporangia are mostly dispersed by wind and there is no active release mechanism per se. However, *P. destructor* sporangia can be vigorously discharged into the air in response to a reduction of relative humidity (Leach 1982; Leach et al. 1982).

This knowledge of sporulation and infection characteristics led, in the mid-1980s, to the development of DownCast, a forecaster for ODM (Jesperson and Sutton 1987; Sutton and Hildebrand 1985). The model provides a dichotomic output (0 or 1). Sporulation of *P. destructor* is predicted when (1) the mean temperature is below 24 °C between 08:00 and 20:00 the previous day; (2) the average night temperature is between 4 °C and 24 °C; (3) the relative humidity is

greater than 95% between 02:00 and 06:00; and (4) no rain should occur after 02:00 (Jesperson and Sutton 1987). This rule-based model is still in use in several growing regions, including Québec and Ontario, but despite its relative accuracy, the authors suggested that it could be improved. Hence, other models such as Onimil, Zwipero, DownCast-deVisser and Milioncast were developed to predict the risk of *P. destructor* sporulation or infection, or both (Battilani et al. 1996; de Visser 1998; Friedrich et al. 2003; Gilles et al. 2004).

Prediction systems are essential to make informed disease management decisions on a short-term basis. This type of decision-making is often referred to as tactical, because it takes place during the cropping seasons with the intention to protect the current crop (Savary et al. 2006). In addition to tactical decisions, disease management must also be based on strategic decisions made at short, middle and very long terms (Savary et al. 2006). Middle-term strategic decisions are defined at the field scale and include crop rotations, cultivar selection and seed treatments, whereas long-term strategic decisions are defined at larger scales in space (region, country, continent) and time (multi-year) (Savary et al. 2006). Moreover, the long-term strategic decision is of most importance, because it allows growers to identify where integrated pest management (IPM) is needed for a particular disease and to anticipate when a given disease will pose a threat to a crop in a given production area (Savary et al. 2006). To prevent or delay the development of plant diseases, it is thus recommended to characterize the disease progress on an annual basis and at multiple spatial and temporal scales (Van der Plank 1963; Cunniffe et al. 2015). Nevertheless, the epidemiology of plant diseases is generally studied over a few consecutive seasons because the available historical data collected over appropriate spatial and temporal scales are limited.

Amongst the epidemiological datasets covering a larger number of seasons, those for potato late blight caused by *Phytophthora infestans* are probably more numerous. In The Netherlands, for example, the analysis of a sequence of 47 potato late-blight epidemics has shown that the presence and level of late blight the previous year, the number of rainy days and relative humidity were important variables allowing for an accurate classification of disease intensity (Zwankhuizen and Zadoks 2002). Similarly, historical data collected in Finland from 1933 to 2002 enabled changes in late blight incidence and epidemics onset to be linked to the increased frequency of rain and warmer temperature at the beginning of the growing season (Hannukkala et al. 2007).

The importance of research on the relationship between climate change and plant diseases through studies conducted over an extended period of time is exacerbated by the speed at which changes are occurring. The latest forecasts regarding global warming suggest a global temperature increase between 1.5 °C and 2 °C over the next 50 years, especially in the northern and southern regions (Hoegh-Guldberg et al. 2018). However, the impact of climate change may vary with the geographic location of the production areas. The distribution and severity of late blight caused by *P. infestans* have increased with global warming and precipitation regime changes in the northern area (Hannukkala et al. 2007), whereas the effect of climate change appears to be limited in tropical and subtropical regions (Sparks et al. 2014).

In the Province of Québec (eastern Canada), the incidence of ODM has been increasing annually since the early 2000s, exceeding 30% of diseased onion fields in 2014. We hypothesize that changes in climatic conditions favor the overwintering of *P. destructor* and, therefore, the duration and precocity of ODM epidemics. Observational data collected over a period of 31 consecutive years are used to: (i) characterize the seasonal distribution patterns of ODM epidemics, (ii) determine if the onset of seasonal epidemics comes earlier and disease incidence is more important and (iii) identify the variables influencing the polyetic ODM development.

4.3 Material and Methods

4.3.1 Onion Production and Study Area

In Québec as in Canada, 80% of the areas dedicated to the production of alliums are dedicated to the production of dry bulb onions, while the remaining 20% are for garlic, leek and green onion. Most of the onion is grown from seed, whereas a small share of the production is grown from transplants or dry sets. Seeding or transplantation is generally performed in late April to early May, when the soil is thawed. Onions are sown about 2–2.5 cm below the soil surface, on 1.8 m wide beds, with double rows spaced 45 cm apart. Even though onions can be produced in various types of soil, the majority of the production is conducted in muck soils.

The study site (Municipalité Régionale de Comté des Jardins-de-Napierville) is located on the south shore of Montreal, surrounded by the Saint-Lawrence River to the north, the Adirondacks to the south and the Richelieu and Châteauguay rivers to the east and west, respectively. The cultivation area (~800 km^2) is between latitude 45°03′ N and 45°14′ N and between longitude

73°42′ W and 73°20′ W. In this area, vegetable crops (mostly onion and lettuce) are grown in muck soil (chernozem), over slightly more than 10,000 ha (Figure 1A).

Climate in the study area is humid continental (Dfb, according to the Köppen classification), characterized by wet and warm summers (i.e., total precipitation from June to August is 293 mm on average, average temperature during the same months is 19.3 °C), cold and snowy winters (average temperature for December–March is −4.72 °C and average snow precipitation is 144.3 cm), with annual mean temperature of 6.6 °C, total precipitation of 799.6 mm and total snow precipitation of 169.3 cm. These climate statistics were compiled and published by the climate monitoring working group of the Ministère de l'Environnement et de la Lutte contre les changements climatiques du Québec (MELCC), for the Hemmingford Four Winds weather station (45°04′21″ N, 73°39′29″ W, 70 m).

4.3.2 Field Sampling, Disease Incidence and Data Aggregation

The dataset analyzed in this study was constructed from the database of PRISME, a Québec bio-monitoring not-for-profit organization (www.prisme.ca). The data were collected by agronomists, crop specialists and summer scouts from 1987 to 2017. Each year, scouting took place once a week from May to August in 122 fields on average per season, which represents ~70% of the total number of onion fields in the region. During scouting, observations on pests and diseases were made on at least 25 onion plants randomly selected in each field, including the presence/absence of ODM (1/0 binary data). The weekly data were then combined spatially to obtain a measure of regional disease incidence (RDI) expressed in percent, i.e., the percentage of infected fields. Thus, data are considered to be counts with an upper bound (Madden et al. 2007). Furthermore, each field was given a value from a severity index (SEVI) ranging from 0 to 3: 0 = no ODM observation, 1 = less than 1%, 2 = between 1% and 5%, and 3 = greater than 5%. The first occurrence of ODM in the region each year was retrieved from the raw data and expressed in days since 1 May. To ensure that the dataset is representative of the region and there is no missing ODM outbreak, the RDI values were validated with experts from the Québec Ministère de l'Agriculture, des Pêcheries et de l'Alimentation and through the Phytosanitary Warning Network (Mario Leblanc, personal communication).

4.3.3 Weather-Related Variables

Data collected by the MELCC climate surveillance group at Hemingford Four Winds (45°04′21″ N, 73°39′29″ W, 70 m) were used in this study. Weather data from 1986 to 2017 were used to define four categories of variables, depending on whether they are: (1) related to sporulation (current growing season); (2) related to infection (current growing season); (3) favoring the production of overwintering inoculum (previous fall); or (4) related to overwintering (previous winter). The first two categories were mostly based on the conditions described by Hildebrand and Sutton (Hildebrand and Sutton 1984c, a, b, 1982; Sutton and Hildebrand 1985). Due to limited information available on the overwintering of *P. destructor*, the third and fourth categories of variables above were built on more general knowledge about peronosporales (Hannukkala et al. 2007; Lehsten et al. 2017; Zwankhuizen and Zadoks 2002; Salgado et al. 2015; Heist et al. 2002; Henderson et al. 2007). All variables used in this study are presented in Table 1.

Variables related to sporulation: These include the number of DownCast daily sporulation periods (DC, the number of days for which DownCast predicts a risk of sporulation) from May to August, and the number of hours with temperature between 4 °C and 24 °C at night (between 20:00 and 6:00) (HT4_24N). Two variables which would prevent sporulation were also considered: the number of hours with temperatures greater than 28 °C at night (NHNT_28) and the number of precipitation events (with at least 0.1 mm of rain) occurring between 20:00 and 6:00 (NSP).

Variables related to infection: The number of hours with relative humidity greater than 90% during the day (NHR_90), the number of hours with temperature between 4 °C and 24 °C during the day (HT4_24IP), the number of precipitation events (characterized by accumulation >0.25 mm) between 6:00 and 12:00 (DSP) and the number of precipitation events during the day (NREIP) are included in this category, as variables potentially enhancing infection. As two variables that would inhibit infection, the average solar radiation (RAD, in J m^{-2}) and the number of hours with temperatures above 28 °C between 6:00 and 20:00 (NHDT_28) are included in the same category.

Variables related to the production of overwintering inoculum: In this study, it was hypothesized that total rainfall during the harvest period (TRH) between 15 August and 15 October was amongst the most important variables influencing the formation of survival structures (oospores). The average and minimum temperatures during the harvest period (ATH and MTH)

were also considered to be enhancing the production of oospores because higher temperature at harvest may lead to a longer period during which oospore production can occur.

Variables related to overwintering: Snow cover (SCOV, in cm), calculated from October 15 the previous year to 15 March the current year, was considered to contribute to the survival of oospores and volunteer plants in soil and cull piles, because snow acts as an insulating layer allowing a certain regulation of the soil temperature. Conversely, off season rainfall (OSRF), also calculated from 15 October to 15 March was considered to affect oospore survival because it may reduce the thickness of the snow cover or lead to the formation of ice. To measure winter severity, we use the Hellmann number (HL), calculated as the sum of average daily temperatures below 0 °C from 15 October to 15 March (Zwankhuizen and Zadoks 2002). The number of hours with temperatures below 0 °C, 5 °C and 10 °C were also used as indicators of coldness. Finally, we used the last RDI value the previous year (DS_PY) and that the year before the previous year (DS_P2Y), as variables of carryover from one or two years to the next.

4.3.4 Data Analysis

As changes in RDI are apparent over the 31 years (Figure 1B), the data were grouped into three periods of 10, 10 and 11 years (period I: 1987–1996, period II: 1997–2006 and period III: 2007–2017), representative of the bimodal distribution of RDI over time. For each of the variables studied, the data distribution was first tested for normality using the Shapiro–Wilk statistic. Since the data were not normally distributed, differences among mean values of the three periods for RDI and weather-related variables (Table 1) were assessed with the Kruskal–Wallis non-parametric test, followed by a Dwass, Steel and Critchlo–Fligner two-sided multiple pairwise comparison test (significance level $\alpha = 0.05$).

Then, for each of the 31 years with enough ODM incidence, the seasonal ODM epidemic was studied by fitting a logistic model to the disease progress curve built on the cumulative RDI, expressed as a proportion of the maximum:

$$Y = \frac{K}{1 + e^{-r*(t - b)}} \quad (1)$$

where the quantity Y is the cumulative RDI, t is the time (in days, for a given season), K is the theoretical upper limit (asymptote) for Y, r is the rate of growth and b is a time offset at which Y is equal to half of the maximum value (mid-time). The 10% epidemic onset (b_{10}), at which Y is equal to 10% of the maximum value, was derived from the fitted model. The goodness-of-fit of the model was assessed by the root mean square error (RMSE) and the coefficient of determination (R^2). A Wilcoxon exact non-parametric test (significance level $\alpha = 0.05$) was performed to determine whether the 10% epidemic onset, mid-time and other model parameter estimates were significantly different between the first and third periods, where the RDI modes are found.

Spearman's rank-based correlation coefficients were computed between weather-related variables and the response variables (RDI, DSI) over the 31 years. The resulting correlation matrix was used in a principal component analysis (PCA), to investigate the relationships between RDI or DSI and the weather-related variables and among the weather-related variables themselves.

Using periods I, II and III as classes, discriminant analyses were performed to identify the variables (excluding RDI and DSI) that influenced the ODM polyetic development differently across the three periods. A discriminant analysis was performed with all the weather-related variables and another with a reduced number of them (i.e., a *sufficient* subset of discriminating variables), retained at the end of a stepwise procedure. The rank-transformed data were used in these analyses. The outputs include the probabilities of classification of the years in the three periods (I, II or III), in an attempt to predict higher or lower ODM occurrence from the weather-related variables.

In the application of multivariate statistical methods (e.g., PCA, discriminant analysis) with time-series data, joining the years in chronological order in biplots facilitates the interpretation of results and such a temporal walk may allow the detection of atypical years within a period in the case of the PCA (Dutilleul and Till 1989). The statistical analyses described above were carried out with procedures NLIN, UNIVARIATE, NPAR1WAY, CORR, PRINCOMP, DISCRIM and STEPDISC from SAS/STAT V9.4 (SAS Institute Inc., Cary, NC, USA).

4.4 Results

Regional disease incidence values in the years 1987–2017 varied from 0 to 33.25% and form a bimodal distribution with one peak in 1990 and the other in 2014 (Figure 1B). The ODM was present in five years out of 10 in period I (mean RDI: 3.59%, *SE* = 2.63), five years out of 10 in period II (mean RDI: 0.11, *SE* = 0.05), and 11 years out of 11 in period III (mean RDI: 9.55, *SE* = 3.17). For the majority of the variables, the null hypothesis of a normal distribution, was rejected according to the Shapiro–Wilk test. The Kruskal–Wallis test shows a significant difference in the mean RDI value among the three periods ($p = 0.0002$). The multiple pairwise comparison test then found a significant difference between periods I and III ($p = 0.0102$) as well as between periods II and III ($p = 0.0003$), but not between periods I and II ($p = 0.5708$) (Table 2).

There are also significant differences among the three periods in the mean value of the weather-related variables, especially the ones related to production and survival of overwintering inoculum. For these, the Kruskal–Wallis test shows significant differences among periods for the average and minimum temperature during harvest and the total rainfall during harvest ($p = 0.0302$, 0.0015 and 0.0008, respectively) (Table 2). In summary, the average and minimum temperatures were significantly warmer while precipitation was more abundant in period III than in period I (Table 2). For the variables related to overwintering, the Kruskal–Wallis test shows significant differences among periods for the disease statuses the previous year and the previous two years ($p = 0.0002$ and 0.0002, respectively), while the Hellmann numbers (HL) suggest warmer winters for period III compared to period I ($p = 0.0084$) (Table 2). For the variables related to sporulation, differences among periods are significant for the number of DownCast periods ($p = 0.0072$), while significant differences are also found for solar radiation in the category of variables related to infection ($p < 0.0001$) (Table 2).

The first ODM outbreak was reported, on average, 75 days after 1 May (Figure 2A). The correlation between year of the survey and time of the first outbreak is negative and significant ($r = -0.700$; $p = 0.0003$) (Figure 2A). More specifically, the first disease outbreak was reported, on average, 93.8, 84.3 and 63.4 days after 1 May, during the first, second and third periods, respectively (Figure 2B). The fitting of logistic models confirms this trend in ODM seasonal epidemics (Figure 3A, Table 3). In addition to earlier disease outbreaks, the 10% epidemic onset

(b_{10}) and mid-time (b) were 15.1 and 11.3 days earlier in period III than in period I (p = 0.0102 and p = 0.0255) (Figure 3B, C).

Through correlations, the PCA was used to evaluate the relationships between weather-related variables and RDI or DSI over the 31 years. The eigenvalues calculated for the correlation matrix correspond to the proportions of dispersion (after standardization) explained by the principal components (PCs), the coefficients of the corresponding eigenvectors indicating the relative importance of each predictor in the composition of the PCs. Taken together, the first three PCs explain 60.3% (PC1: 28.9%, PC2: 20.3% and PC3: 11.1%) of the total dispersion (Table 4). The eigenvectors of the first principal component had values ranging from −0.310 to 0.355, with the highest and lowest value corresponding to disease incidence the previous year (DS_PY) and solar radiation (RAD), respectively. The eigenvectors of the second principal components had values ranging from −0.352 to 0.394, with the highest and lowest value corresponding to the number of hours with temperature between 4 °C and 24 °C during the day (HT4_24IP) and the number of hours with relative humidity greater than 90% (NHR_90), respectively (Table 4). While PC1 was largely associated with variables related to overwintering, PC2 was more related to temperature and relative humidity associated with infection and sporulation (Table 4).

Graphically and accordingly, the RDI and DSI vectors in the PC2-PC1 biplot go in the same direction as those for disease status the previous year (DS_PY) and the previous two years (DS_PY2), minimum temperature at harvest (MTH) and number of morning precipitation events (DSP), whereas the solar radiation (RAD) vector points in the opposite direction (Figure 4A). In the year space (Figure 4B), it is possible to follow the chronology of events along PC1, with the period I years on the left, the period III years on the right and the period II years in-between; PC2 tends to show fluctuations among years within a period.

When using all the weather-related variables in the discriminant analysis, only 50% of the years belonging to period I, 80% of the years belonging to period II and 81.8% of the years belonging to period III are correctly classified (Figure 5A). Overall, the achieved classification accuracy is 70.6%. At the end of the stepwise procedure, five variables (MTH, DS_PY, HL, TRH and RAD) were retained as a sufficient discriminating subset (Table 5). Using these five variables, 90%, 90% and 100% of the years belonging to the first, second and third ODM periods are correctly classified (Figure 5B), and the achieved overall classification accuracy is 93.5%.

4.5 Discussion

Although the local conditions for *P. destructor* infection and sporulation were thoroughly studied in the 1980s (Hildebrand and Sutton 1980, 1984c, a, b, 1982; Jesperson and Sutton 1987; Leach et al. 1982; Sutton and Hildebrand 1985), the processes underlying ODM seasonal establishment are poorly understood. Results of the analyses of scouting data collected from 1987 to 2017 in our study allowed a quantitative description of the ODM epidemics in the long term over time and at a regional scale. The epidemics occurring during period III (2007–2017) were more severe than those during period I (1987–1996) and were characterized by the first ODM observation dates that were earlier in the year (30.4 days on average). The first disease observation date is a valuable indicator of change in disease epidemiology but may not be representative of the seasonal establishment of the disease in a given area. For this reason, we fitted models to regional disease progress curves to calculate and compare 10% epidemic onset and mid-time for the three ODM periods. Results of the non-linear regression analysis (logistic model fitting) are consistent with those for the first observation dates, as the 10% epidemic onset and mid-time are, respectively, 15.1 and 11.3 days earlier in 2007–2017. The incidence of ODM during the transition between the second and third periods was too low for seasonal disease establishment, so that no logistic model was fitted for the corresponding years. However, these observations are of interest, as they heralded a shift in the polyetic ODM development. A similar trend was observed for potato late blight caused by *P. infestans* in Finland. Using a historical dataset from 1933 to 2002, Hannukkala et al. (2007) showed that late blight epidemics started two to four weeks earlier in the years 1996–2002 than in 1933–1962. This trend was later confirmed for the 2002–2012 period (Lehsten et al. 2017), and a similar trend was also reported in Sweden for 1983–1992 compared to 1993–2012 (Wiik 2014).

As for other *Peronosporaceae*, *P. destructor* has long been thought to be a periodically introduced oomycete because of multiyear ODM epidemics followed by multiyear absence of the epidemic (LaMondia and Aylor 2001). For these pathogens, atmospheric transport of sporangia is considered to play a major role in their long-distance spread. Thus, the presence of periodically introduced oomycetes in northern latitudes largely depends on sporangia dispersal from outbreaks occurring in the South (Aylor 2017; Aylor 2003; Ojiambo et al. 2015). Their sporadic presence in northern areas may be the result of a lack of local production of oospores or simply because they

cannot survive the rigor of winters beyond a certain latitude. This is the case of *Pseudoperonospora cubensis*, which is not known to overwinter in the field above 30° latitude North (Ojiambo et al. 2015; Ojiambo and Holmes 2010; Cohen et al. 2015). Long-distance dispersal is subjected to several factors (e.g., presence of susceptible hosts, anthropogenic trades), but it is reasonable to expect that the closer the overwintering source, the earlier and the more frequent and severe the epidemics will be. Our results are supportive of the hypothesis of a continuous shift poleward of plant pathogens, possibly because of global climate change (Bebber et al. 2013). While *P. destructor* mainly spreads by aerial dispersal, climatic conditions are likely to determine the subsequent establishment of the disease in a given area. Hence, our results point to an adaptation of the pathogen with respect to the production and survival of overwintering inoculum under the south-western latitudes of Québec.

An increasing number of studies provide ever more accurate predictions of the effects of climate change on plant diseases (Sparks et al. 2014; Anderson et al. 2004; Francesca et al. 2006; Savary et al. 2011; Duku et al. 2016; Caubel et al. 2014; Launay et al. 2014), but studies remain limited and constrained by the availability of data (Mike and Pautasso 2008; Garrett 2013). Moreover, when available, the observations may come from several sources and in different types (discrete, categorical or continuous). Thus, it is often impossible to choose the appropriate scale (field, farm, county, country or continent). In our study, even though the original observations were made at the plant level, there is one line of data per field per date in the database. It follows that the fields are considered as the units of infection and the epidemics are studied at two nested time scales (i.e., the year and the day within a year), enabling a smooth shift to multi-year regional disease level (Gilligan 2008). In their long-term analysis of potato late blight epidemics, Zwankhuizen and Zadoks (2002) had to merge qualitative and quantitative observations from several sources (annual reports of plant protection service, journal articles and unpublished data) and accept some arbitrariness of classification, to finally work at an aggregation level of years and countries in time and space (Zwankhuizen and Zadoks 2002). To investigate the poleward shift of pests and plant pathogens in response to global warming, Bebber et al. (2013) used data from the CABI (Commonwealth Agricultural Bureau) distribution maps which were also aggregated at the country level.

The study of the polyetic development of plant diseases and their interactions with climate change is highly relevant in a context of changing agricultural landscape. As a result, diseases may emerge where they were non-existent and the frequency of occurrence of existing diseases may increase, but also the status of plant pathogens known to be periodically introduced may change and become endemic. Among the factors that can influence the distribution of plant pathogens or the magnitude of seasonal epidemics, temperature and precipitation regimes are of most importance. For some diseases, it is realistic to expect that increasing temperature favors overwintering and increases the number of seasonal disease cycles, while changes in precipitation regime might enhance (or disfavor) infection processes (Garrett 2013). Our PCA results suggest that regional disease incidence (RDI) was mostly influenced in a season by precipitation (NSP, DSP), but not by diurnal or nocturnal temperature (NHNT_28, HT4_24N, NHDT_28, HT4_24IP). Because *P. destructor* is known to be more aggressive at lower temperatures (i.e., favorable temperatures are between 4 °C and 24 °C) (Hildebrand and Sutton 1982), whereas sporangia germination is much lower above 26 °C and completely inhibited above 28 °C (Jesperson and Sutton 1987), it was expected that these temperature variables would have been important. The PCA results also suggest that regional disease incidence was influenced by the variables related to the production of oospores and their survival. The disease status the previous year and the previous two years (DS_PY, DS_P2Y) appear to be an important source of carryover, mainly because the larger the number of ODM cases, the higher the probability of oospore production. Warmer temperature at harvest time the previous year (MTH) was also shown to be an important factor. Higher temperatures towards the end of the growing season is thought to give the pathogen additional time to form oospores, possibly in larger quantity.

The meteorological variables that vary the most among the three periods are related to oospore production and survival. The mean and minimum temperatures during harvest (ATH and MTH) were higher, precipitation during harvest (TRH) was more abundant, and the winter was less severe in period III than in period I. Using the subset of five variables retained at the end of the stepwise procedure (i.e., MTH and TRH: temperature and precipitation at harvest the previous fall; HL: severity in winter; RAD: solar radiation and DS_PY: disease incidence the previous year), the discriminant analysis results improved considerably from 70.6% to 93.5% correct classification. Except for solar radiation, which is known to affect negatively the viability of oomycete sporangia (Hildebrand and Sutton 1984b; Leach et al. 1982; Skelsey et al. 2018), the

variables influencing RDI the most are variables related to the production and survival of overwintering structures.

The role of oospores in the epidemiology of ODM is under-documented. It is known that *P. destructor* produces oospores in natural conditions and these oospores can be long-lasting in the soil (McKay 1957; McKay 1937). In an experiment conducted over 25 consecutive years, McKay (1957) found that oospores were able to germinate up to 25 years after their production. In the same study, it was shown that oospores needed an incredibly long maturation period, as the first observation of germination occurred after four years and reached the maximum germination rate after seven years (McKay 1957). Although it would be surprising that this oomycete produced oospores adapted for such a long-term survival for no apparent reason, their potential as a source of primary inoculum remains ambiguous. There is no clear evidence that oospores present in soil can lead to infection (McKay 1957; Cook 1932; Palti 1989). Alternatively, systemically infected tissues (mainly infected immature bulbs) can be an important source of primary inoculum (Hildebrand and Sutton 1980; Palti 1989; Abd-Elrazik and Lorbeer 1980). Whether it is from oospores or infected crop residues, the results obtained in this study suggest that variables favoring the production and survival of overwintering structures may play an important role in seasonal ODM epidemics.

The assumption of overwintering of *P. destructor* inoculum entails changes in tactical and strategic decision-making. The information on overwintering inoculum could become an important component of tactical decisions, for example, to plan the deployment of spore-trapping networks. In addition, the disease status the previous year and the harvest and winter weather conditions can also be used as baseline information for risk prediction models during the current year. The implication of this assumption is also meaningful for short-term strategic decisions because the presence of inoculum in soils is likely to influence the planning of crop rotations and the selection of cultivars and cropping systems (Savary et al. 2006).

In general, the use of observational data implies the acceptance of greater uncertainty in the data, the potential introduction of bias due to multiple data sources, and the acceptance of correlations instead of understanding causation (Garrett 2013; Bebber et al. 2013). However, observational data and their analysis can reveal significant trends and influence long-term tactical decisions. Our main results are that ODM epidemics in south-western Québec tend to come earlier

and more frequently, and that the increased disease incidence is linked to weather variables related to overwintering and disease carryover from one growing season to the next. Either there is production and survival of overwintering inoculum at the latitudes of south-western Quebec, or the production areas where survival is possible are closer than before. Finally, this study also contributes to long-term tactical decisions by highlighting the need for more research on overwintering and for the development of molecular-based tools enabling the monitoring of initial and secondary inoculum.

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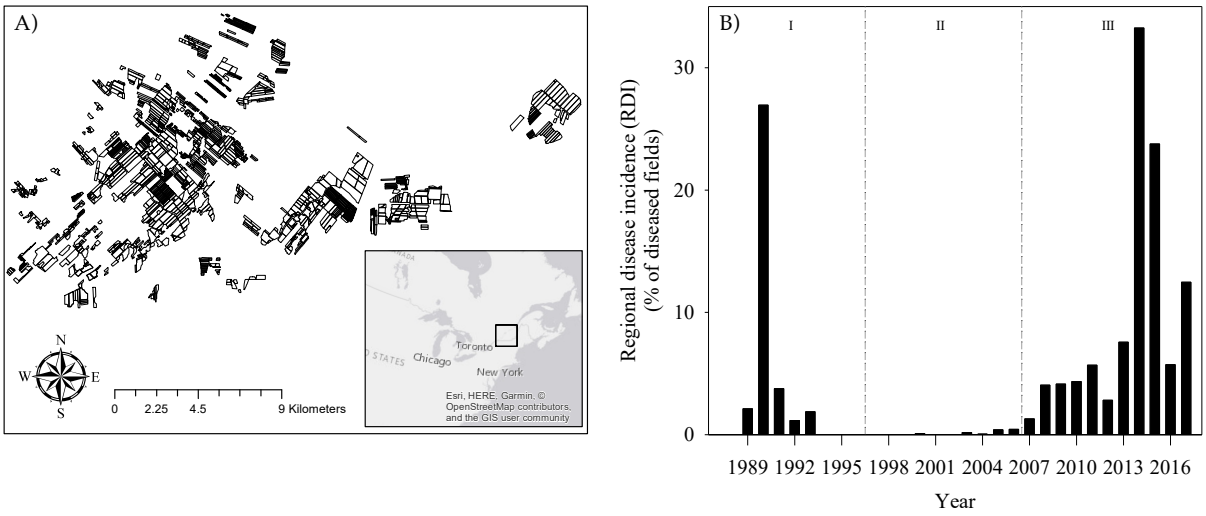

Figure 4-1. Description of fields locations and disease incidence. Location of onion fields included in the study over the years (A) and regional disease incidence (RDI) of onion downy mildew (ODM) epidemics observed in the Napierville County for each of the 31 years of the dataset at the end of the growing season (B).

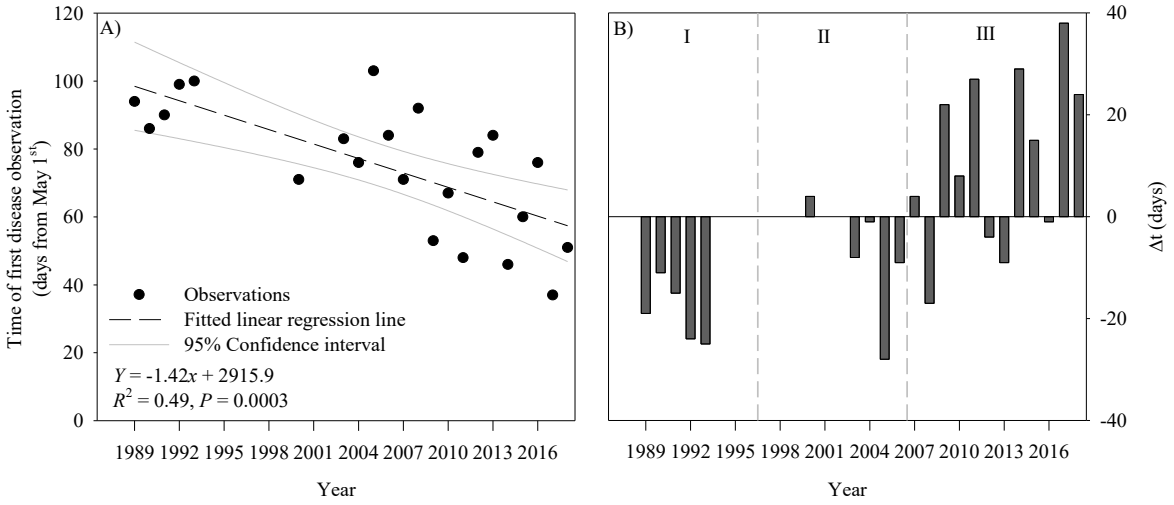

Figure 4-2. Changes in first onion downy mildew observation date.Plots against Year for (A) the earliest time of ODM observation from 1 May, with linear regression analysis results, and (B) the difference between the average first observation date and the first observation date for each year during which ODM was observed.

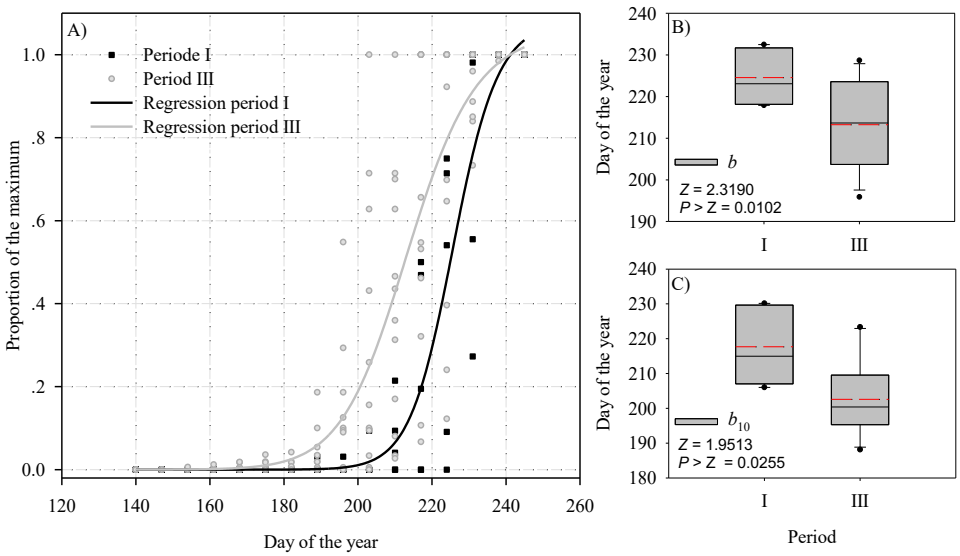

Figure 4-3. Disease progress curves for the first and third ODM periods. Box plots of (B) model parameter estimate *b*, which represents the time (in days) at which seasonal ODM incidence is half of its maximum value (mid-time) and (C) b_{10}, the time (in days) when seasonal ODM incidence is equal to 10% of the maximum value (10% epidemic onset). Period I include the years 1987–1996, and period III, 2007–2017. In each box plot, the horizontal black lines, from bottom to top, represent the 10th, 25th, 50th (median), 75th and 90th percentiles, the red dashed line represents the mean and a black circle represents an outlier. The Z statistic and associated *p*-value are for the Wilcoxon exact test (significance level $\alpha = 0.05$).

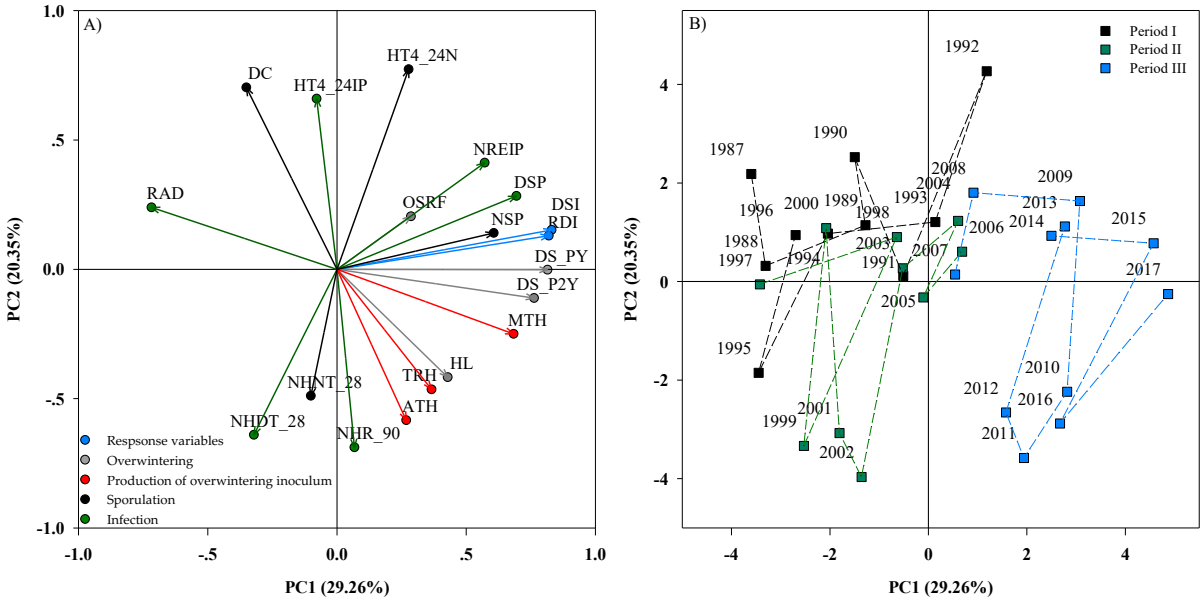

Figure 4-4. Principal component analysis (PCA) biplots obtained by using the first two principal components (A) in the variable space and (B) in the year space, where consecutive years are linked to allow following the temporal walk.

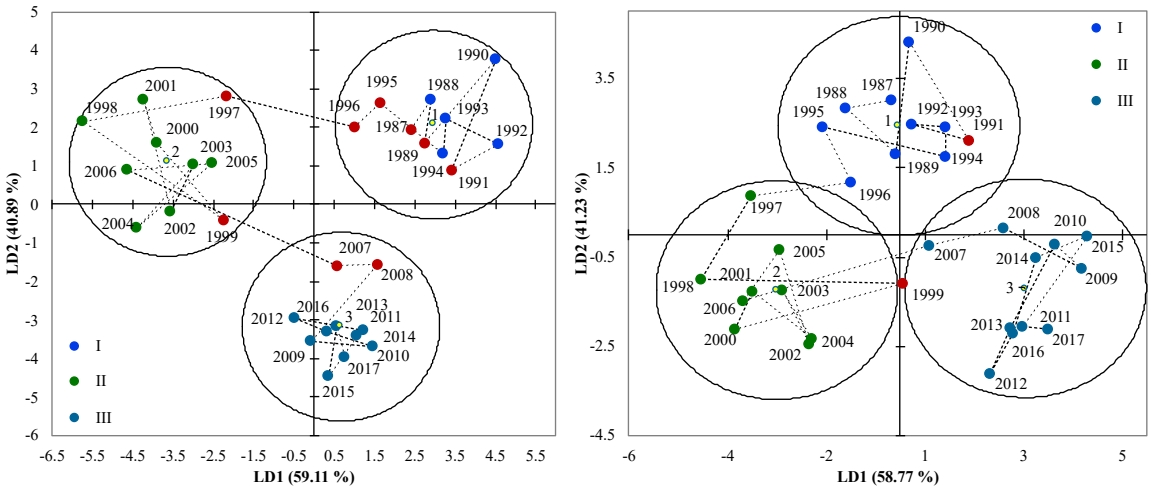

Figure 4-5. Discriminant analysis results presented graphically in biplots of the second linear discriminant variable (LD2) against the first (LD1). The blue, green and turquoise points represent the years correctly classified in periods I, II and III, respectively. The red points indicate the years that are misclassified. A) All the weather-related variables are used, and the overall correct classification rate is 70.6%. B) The discriminating subset of five variables (MTH, DS_PY, HL, TRH, RAD) found to be sufficient at the end of the stepwise procedure is used: 93.5% of the years are correctly classified within the three ODM periods.

Table 4-1. Description of the variables considered in this study.

Category	Variables	Definition
Dependent variables	RDI	Regional disease incidence (% of infected fields)
	DSI	Disease intensity (from 0 to 3)
Related to sporulation (Calculated from 1 May to 15 August)	DC	Number of DownCast sporulation periods
	NSP	Number of night precipitation events (between 24:00 and 6:00)
	HT4_24N	Number of hours with temperature between 4 °C and 24 °C (between 20:00 and 6:00)
	NHNT_28	Number of hours with temperatures above 28 °C (between 20:00 and 6:00)
Related to infection (Calculated from 1 May to 15 August)	RAD	Average solar radiation (J m^{-2})
	DSP	Number of morning precipitation events (between 7:00 and 12:00)
	NREIP	Number of precipitation events during infection period
	NHR_90	Number of hours with relative humidity above 90%
	HT4_24IP	Number of hours with temperature between 4 °C and 24 °C (between 6:00 and 20:00)
	NHDT_28	Number of hours with temperatures above 28 °C (between 6:00 and 20:00)
Related to production of overwintering inoculum (Calculated from 15 August to 1 October the previous year)	ATH	Mean temperature during harvest (°C)
	MTH	Minimum temperature at harvest (°C)
	TRH	Total rainfall at harvest (mm)
Related to overwintering (HL was calculated from November to March)	SCOV	Snow cover (mm)
	DS_PY	Final RDI previous year
	DS_P2Y	Final RDI the year before previous year
	HL	Hellmann number (sum of average daily temperatures below 0 °C) [23]
	OSRF	Off growing season rainfall (mm)

Table 4-2. Mean values of regional disease incidence (RDI) and weather-related variables, and results of the Kruskal–Wallis test (observed value of the test statistic and corresponding probability of significance).

Variables *	Period **			χ^2	p
	I	II	III		
RDI	3.6 [a]	0.1 [a]	9.6 [b]	17.20	0.0002
DC	33.7 [b]	22.0 [a]	20.18 [a]	9.87	0.0072
NSP	52.6	66.9	80.5	4.73	0.0941
HT4_24N	1245.3	1207.5	1227.7	0.75	0.6867
NHNT_28	0.4	0.3	0.6	0.01	0.9945
RAD	15.94 [b]	15.77 [b]	12.32 [a]	20.45	<0.0001
DSP	79.3	82.6	107.5	5.33	0.0699
NREIP	33.4	34.1	47.6	4.11	0.1284
NHR_90	413.0	486.6	468.6	5.23	0.0732
HT4_24IP	451.5	447.2	446.5	0.94	0.624
NHDT_28	45.2	41.9	41.5	0.11	0.9463
ATH	17.30 [a]	18.42 [a,b]	18.81 [b]	6.99	0.0302
MTH	7.28 [a]	9.5 [b]	10.5 [b]	13.05	0.0015
TRH	42.6 [a]	90.9 [b]	104.82 [b]	14.15	0.0008
SCOV	154.6	146.4	158.7	0.06	0.9683
DS_PY	3.6 [a]	0.1 [a]	8.5 [b]	16.92	0.0002
DS_P2Y	3.6 [a]	0.1 [a]	7.99 [b]	16.76	0.0002
HL	−111.7 [b]	−98.97 [a,b]	−94.46 [a]	9.56	0.0084
OSRF	330.1	289.7	298.4	0.12	0.9424

* Mean values with different letters within the same row are significantly different according to the Dwass, Steel and Critchlo–Fligner two-sided multiple pairwise comparison test (significance level $\alpha = 0.05$).

** Period I include the years from 1987 to 1996, period II from 1997 to 2006, and period III from 2007 to 2017.

Table 4-3. Summary of the logistic model (Equation (1)) fitting results for each of the 31 years (1987–2017) with enough ODM incidence.

Year	Parameter [a]	Estimate	*SE*	Approximate 95% Confidence Limits		Model *p*-Value	Year	Parameter	Estimate	*SE*	Approximate 95% Confidence Limits		Model *p*-Value
	k	1.024	0.024	0.972	1.077			*k*	1.019	0.036	0.941	1.098	
1989	*r*	0.187	0.017	0.150	0.225	0.0001	**2010**	*r*	0.151	0.024	0.099	0.204	0.0001
	b	217.900	0.594	216.600	219.200			*b*	202.800	1.260	200.100	205.500	
	k	1.028	0.017	0.990	1.065			*k*	1.040	0.049	0.935	1.145	
1990	*r*	0.274	0.021	0.228	0.321	0.0001	**2011**	*r*	0.141	0.026	0.084	0.198	0.0001
	b	223.100	0.326	222.400	223.800			*b*	206.400	1.594	202.900	209.800	
	k	1.020	0.022	0.973	1.068			*k*	1.000	0.001	1.000	1.000	
1991	*r*	0.214	0.020	0.171	0.257	0.0001	**2012**	*r*	2.021	0.013	1.994	2.048	0.0001
	b	218.400	0.513	217.300	219.500			*b*	195.900	1.32×10^{-9}	195.900	195.900	
	k	1.012	0.025	0.959	1.066			*k*	1.011	0.017	0.975	1.047	
1992	*r*	0.663	0.196	0.240	1.086	0.0001	**2013**	*r*	0.412	0.032	0.344	0.480	0.0001
	b	232.500	0.496	231.400	233.600			*b*	228.700	0.255	228.100	229.200	
	k	1.000	0.001	1.000	1.000			*k*	1.072	0.053	0.957	1.187	
1993	*r*	1.993	0.008	1.976	2.009	0.0001	**2014**	*r*	0.164	0.022	0.116	0.211	0.0001
	b	230.900	0.001	230.900	230.900			*b*	224.800	1.089	222.500	227.200	
	k	0.954	0.030	0.891	1.018			*k*	1.057	0.040	0.970	1.144	
2006	*r*	0.630	0.340	−0.104	1.364	0.0001	**2015**	*r*	0.128	0.015	0.095	0.160	0.0001
	b	201.400	0.999	199.200	203.500			*b*	213.400	1.176	210.900	216.000	
	k	1.005	0.018	0.966	1.043			*k*	1.006	0.021	0.961	1.051	
2007	*r*	0.420	0.060	0.289	0.550	0.0001	**2016**	*r*	0.560	0.093	0.359	0.760	0.0001
	b	208.000	0.423	207.100	208.900			*b*	226.000	0.406	225.100	226.900	
	k	1.014	0.016	0.979	1.050			*k*	1.121	0.041	1.032	1.210	
2008	*r*	0.262	0.023	0.213	0.312	0.0001	**2017**	*r*	0.098	0.007	0.083	0.112	0.0001
	b	213.900	0.400	213.100	214.800			*b*	219.900	1.131	217.400	222.300	
	k	1.025	0.032	0.956	1.094								
2009	*r*	0.150	0.015	0.118	0.182	0.0001							
	b	217.900	0.831	216.100	219.700								

[a] Parameters of the logistic model used in this study.

Table 4-4. Composition of the eigenvectors (as linear combinations of variables) for the first three principal components in the PCA.

Categories	Variables	Principal Components		
		1	2	3
Related to sporulation	DC	−0.1501	0.3595	−0.1119
	NHNT_28	−0.0422	−0.2494	0.2626
	HT4_24N	0.1225	0.3935	−0.0859
	NSP	0.258	0.0695	0.3646
	DSP	0.2905	0.1403	0.4098
Related to infection	RAD	−0.3104	0.1242	0.187
	NHDT_28	−0.1389	−0.3254	0.0754
	HT4_24IP	−0.0299	0.3353	−0.2817
	NHR_90	0.0295	−0.3518	0.0737
	NREIP	0.2409	0.2085	0.4248
Related to production of overwintering inoculum	ATH	0.1088	−0.2944	−0.1261
	TRH	0.1538	−0.2353	−0.1137
	MTH	0.2930	−0.1287	−0.1455
Related to overwintering	OSRF	0.1171	0.1021	0.3673
	HL	0.1821	−0.2124	0.0499
	DS_P2Y	0.3175	−0.059	−0.1326
	DS_PY	0.3418	0.0015	−0.1674
	RDI	0.3499	0.0668	−0.1576
	DSI	0.3549	0.0776	−0.2156
	Cumulative variation accounted for (%)	28.93	49.23	60.25

Table 4-5. Weather-related variables retained in the stepwise discriminant analysis procedure, applied to identify the smallest sufficient set of discriminating variables for periods I, II and III, and the corresponding statistics.

Variables [a]	*F*-Value [b]	*p* > *F*	Partial R^2 [b]	Wilks' Lambda [c]	*p* < Lambda
RAD	29.570	<0.0001	0.6787	0.3213	<0.0001
DS_PY	10.310	0.0005	0.4331	0.1821	<0.0001
MTH	15.750	<0.0001	0.5479	0.0823	<0.0001
TRH	8.370	0.0016	0.4011	0.0493	<0.0001
HL	2.380	0.1145	0.1652	0.0412	<0.0001

Connecting text for chapter 5

The results presented in Chapter 4 suggested that regional disease incidence is primarily influenced by temperature and precipitation at harvest the previous fall, winter hardiness, solar radiation, and disease incidence the previous year. Together, these results suggested that *P. destructor* can now overwinter in northern latitudes and potentially in southwestern Quebec. Thus, if the pathogen can overwinter locally, it should be possible to detect the inoculum in the environment. However, proper detection tools were not yet available to accomplish this. Chapter 5, therefore, describes the development of a reliable and sensitive hydrolysis probe-based assay multiplexed with an internal control to quantify soil- and airborne inoculum of *P. destructor*. Through the validation process, 290 soil samples were collected in the spring to avoid sampling short-term survival structures, and of these, nearly 10% had a quantifiable amount of inoculum. These results further supported the hypothesis of overwintering under Northern climates.

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Minor modifications were made for uniformity and to conform to the McGill University thesis guidelines.

5 Monitoring of Peronospora destructor Primary and Secondary Inoculum by Real-Time qPCR

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5.1 Abstract

Onion Downy Mildew (ODM), caused by *Peronospora destructor*, is a serious threat for onion growers worldwide. In southwestern Québec, Canada, a steady increase in occurrence of ODM is observed since the mid 2000s. On onion, *P. destructor* can develop local and systemic infections producing numerous sporangia which are acting as initial inoculum locally, but also for neighboring areas. It also produces oospores capable of surviving in soils and tissues for a prolonged period of time. A recent study showed that ODM epidemics are strongly associated to weather conditions related to production and survival of overwintering inoculum, stressing the need to understand the role of primary (initial) and secondary inoculum. However, *P. destructor* is an obligate biotrophic pathogen which complicates the study of inoculum sources. This study aimed at developing a molecular assay specific to *P. destructor* allowing its quantification in environmental samples. In this study, a reliable and sensitive hydrolysis probe-based assay multiplexed with an internal control was developed on the internal transcribed spacer (ITS) region to quantify soil- and airborne inoculum of *P. destructor*. The assay specificity was tested against 17 isolates of *P. destructor* obtained from different locations worldwide, other Peronosporales and various onion pathogens. Validation with artificially inoculated soil and air samples suggested a sensitivity of less than 10 sporangia g^{-1} of dry soil and 1 sporangium m^{-3} of air. Validation with environmental air samples shows linear relationship between microscopic and real-time qPCR counts. In naturally infested soils inoculum ranged from 0 to 162 sporangia equivalent g^{-1} of dry soil, which supported the hypothesis of overwintering under Northern climates. This assay will be useful for primary and secondary inoculum monitoring, to help characterize ODM epidemiology and could be used for daily tactical and short-term strategic decision-making.

5.2 Introduction

Among all vegetables grown in Canada, onion (*Allium cepa* L.) ranks third behind carrot and tomato, with a farm gate value of nearly CAD$95 million (Mailvaganam 2017). In Canada, 80% of the onion production is grown in the muck soils (chernozem) of Ontario and southwestern Québec. Typically, onions are sown at the end of April and harvested in September, with yields up to 60 tons per hectare. During the growing season, the crop is highly susceptible to many airborne diseases including botrytis leaf blight (*Botrytis squamosa*), Stemphylium leaf blight (*Stemphylium vesicarium*) and onion downy mildew (*Peronospora destructor*). The latter being one of the most preoccupant, with yield losses reaching up to 75% (Develash and Sugha 1997; de Araújo et al. 2017). The disease was reported in almost all production area but is more severe in temperate climates (Develash and Sugha 1997; de Araújo et al. 2017; Gerald and Obrien 1994; Yarwood 1943). In Southwestern Québec, the disease is in progression since the mid 2000s with regional disease incidence reaching up to 33% of onion fields diseased in 2014 (Van der Heyden et al. 2020).

Onion Downy mildew (ODM) is caused by the oomycete *P. destructor* (Berk.) Caspary. The firsts symptoms appear as pale green to yellow elongated ovoid lesions harboring violet-grey sporulation, with infected leaves rapidly decaying, turning from green to pale green to yellow which eventually collapses. Affected plants eventually dry standing, affecting yields and storage potential. The disease often begins in small foci and spreads rapidly to surrounding areas. Infection by *P. destructor* requires a continuous period of leaf wetness and temperature between 5 and 27°C, with optimum temperatures being between 10-18°C (Hildebrand and Sutton 1984a). After infection, symptoms and new sporulation appears following a latency period of 13 to 17 days, depending on temperatures (Hildebrand and Sutton 1984b). The sporulation of *P. destructor* is a process dependent on photoperiod (Yarwood 1937), requiring relative humidity greater than 95% and night temperature between 4°C and 25°C (Hildebrand and Sutton 1982). At maturity, sporangia can be vigorously discharged into the air in response to a reduction of relative humidity (hydrostatic movement) but the dispersal is mostly wind-driven (Leach 1982; Leach et al. 1982). Hence, daily airborne concentrations of *P. destructor* sporangia generally follow a unimodal distribution, beginning early in the morning, about 1.5h after sunrise, and reaching their maximum as temperature increases and relative humidity decreases (Hildebrand and Sutton 1982).

Although *P. destructor* sporangia are largely responsible for seasonal development and spatial dispersion of the disease (secondary inoculum), information on sources of primary inoculum is limited. It was suggested that *P. destructor* can survive as mycelium on infected onion sets and volunteer plants (Hildebrand and Sutton 1980). However, like other oomycetes, *P. destructor* also reproduces sexually as oospores, although it is not known if survival in soil and crop debris is due to homothallism, heterothallism or both (Judelson 2008; Palti 1989). Actually, *P. destructor* oospores has been shown to remain viable for up to 25 years (McKay 1957), a record for the genus (Judelson 2008). In a recent study, Van der Heyden et al. (2020) showed that ODM epidemics in Southwestern Québec were more frequent over the last decade and occurs earlier compared to compared to earlier periods (1987-1996 and 1997-2006). The results of that study also suggested that ODM incidence is closely related to weather variables associated to the production and survival of overwintering structures. These findings emphasized the importance of inoculum sources and the need to better understand the role of both primary and secondary inoculum in the ODM epidemics.

Being able to detect and quantify inoculum is critical for effective disease management. Moreover, the study of primary inoculum sources (oospores) is difficult without appropriate detection tools. The only assay available so far for the detection of *P. destructor* sporangia is based on a monoclonal antibody used in a lateral flow device (Kennedy and Wakeham 2008). However, although its usefulness has been demonstrated for detection in planta, the assay has a detection limit of about 500 sporangia, which might be too high for adequate disease risk estimation. In addition, monoclonal techniques are time consuming and less cost-effective than PCR-based methods. The measurement of plant pathogen inoculum in different epidemiological substrates (air, soil, seed, water, plant) can be performed through classical methods such as direct microscopic observations or by direct plating on selective culture media (Hendrix and Campbell 1973; Lacey and West 2006). These methods have been proven to be limiting for airborne and soil-borne diseases mainly because of the difficulties to identify pathogens at the specie level (Bilodeau 2011; Lievens et al. 2005; West et al. 2008). Besides, culture dependent approaches rely on the capacity of the pathogen to grow on artificial media and therefore are not suitable for obligate biotrophic pathogens such as *P. destructor*.

Molecular approaches, especially polymerase chain reaction (PCR), can circumvent many of these limitations. In general PCR-based methods are faster, sensitive, accurate and more

importantly, they allow for detection of non-cultivable plant pathogens. These methods include endpoint PCR, RFLP-PCR, microarrays, sequencing, pyrosequencing, real-time qPCR (SYBR green or probe based) and next generation sequencing. Among the downsides of PCR-based approaches, they do not allow discrimination between viable and non-viable or between infectious and non-infectious propagules. However, for many reasons, probe-based real-time qPCR is considered to be the golden standard for specific pathogen quantitation: it takes significantly less processing time than the other PCR methods or sequencing; it is more specific than endpoint PCR or SYBR green real-time qPCR and it can be multiplexed, which allows the use of internal controls to measure reaction efficiency/inhibition (Crandall et al. 2017).

Research and development of real-time qPCR-based markers were conducted for several species belonging to the *Phytophthora* genus, especially following the finding of the genus specific *atp9-nad9* loci (Bilodeau et al. 2014; Miles et al. 2017). Several assays were also developed for species belonging to the genus *Pythium*, although some species are indistinguishable from one another using usual barcodes (Schroeder et al. 2013; Tambong et al. 2006). However, despite their economic importance in agriculture, only few assays were developed for other Peronosporales such as *Bremia lactucae* (Kunjeti et al. 2016), *Peronospora effusa* and *P. schachtii (Klosterman et al. 2014)* and *P. arborescens (Montes-Borrego et al. 2010)*. These PCR-based assays were extremely sensitive (10 to 100 gene copy number) and specific to the target species.

The availability of a sensitive and specific molecular tool for detecting the primary and secondary inoculum of *P. destructor* in the environment is critical in the acquisition of knowledge of ODM epidemics and the pathogen's ability to overwinter. Therefore, this study aimed at developing a specific multiplex real time hydrolysis probe qPCR assay for *P. destructor* identification and quantification in soil or air samples. The specificity and sensitivity of the developed assay was validated on fungal and oomycetes DNA, soil and air samples artificially and naturally infested.

5.3 Materials and Methods

5.3.1 Isolates collection.

Peronospora destructor isolates were collected on diseased onions from fields primarily located in south-western Québec (MRC Des Jardins de Napierville, 73° 24' to 73° 37' West, 45°

10' to 45° 16' North), and on a lesser extent from other regions including France, California and Ontario (Canada) (Table 1). Other Peronosporales isolates were collected from diseased plants in commercial fields, greenhouses and gardens or obtained from collaborators. Samples consisted of sporangia harvested from the leaf surface using a BBL culture swab (Fisher Scientific, Mississauga, ON. Canada) and placed in a 2.0 ml micro-centrifuge tube containing 300 ul of 100% isopropanol. Sporangia suspensions were kept at -20ºC until DNA extraction. Non target fungal species were also collected from diseased onion plants and obtained from the Quebec provincial diagnostic laboratory (Laboratoire d'expertise et de diagnostic en Phytoprotection, MAPAQ, Qc, Canada).

5.3.2 DNA extraction and sequencing.

Total DNA was extracted from sporangial suspension using the DNA extraction procedure described in (Carisse et al. 2009). Briefly, to disrupt the sporangia wall, 80 ul of the sporangial suspension was homogenized in 2 ml micro-centrifuge tube containing 100 mg of 425-600 µm glass beads (Sigma-Aldrich Canada Ltd., ON, Canada), in a Fastprep instrument (MP Biomedicals, OH, USA) for 40 sec at a speed setting of 6.0 m s^{-1}, followed by a boiling step (20 min at 105 °C) in 300 µl of a resuspension buffer containing 5% chelex100 extraction buffer (Bio-Rad Laboratories, ON, Canada) and a final centrifugation at 4 °C for 5 min at 15 000 x g. Fungal DNA from cultured fungi was obtained using the FastDNA Spin Kit (MP Biomedicals, OH, USA) with the CLS-Y buffer, according to the manufacturer instructions. Following DNA extraction, the high copy numbers internal transcribed spacer (ITS) region was amplified by PCR using the primers DC6 (specific for Pythiales and Peronosporales) and ITS4 (Cooke et al. 2000; White et al. 1990). The quality and quantity of extracted DNA were assessed using a Nanodrop lite instrument (Thermo Fisher Scientific, ON, Canada). The PCR contained 1x Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolab, MA, USA), 500 nM of each primer and 5 µl of template DNA. All PCR were conducted in a SureCycler 8800 (Agilent Technologies Canada Inc., ON, Canada) in a final volume of 30 µl, and the cycling conditions were: 2 min at 98 °C followed by 35 cycles of 10 sec at 98 °C, 15 sec at 60 °C and 30 sec at 72 °C, and a final extension step of 10 min at 72 °C. PCR products were purified using the PureLink PCR purification kit (Thermo Fisher Scientific) and sent to the Centre de Recherche du CHUL/CHUQ at Laval University for Sanger sequencing. The DNA sequences obtained for *P. destructor* and other Peronosporales were

aligned with a subset of non-redundant sequences selected from GenBank, using the MAFFT alignment method available in Geneious V9.1.5 (Katoh et al. 2002).

5.3.3 Primers and probe design.

The DNA alignment was first examined to find regions highly conserved amongst *P. destructor* isolates and bearing the most difference from other species. The primers and hydrolysis probe were designed using Primer Express (V3.0.1) so that nucleotides specific to *P. destructor* were located at the 3' end of the primers and in the middle of the hydrolysis probe (Bilodeau 2011). Moreover, a difference of 10°C between the primers melting temperature (Tm) and the probe Tm was respected. A first evaluation of primers and probe specificity was conducted in-silico by blasting the primer sequences against a non-redundant GenBank dataset with parameters set for the identification of short, nearly exact matches. Secondly, a neighbor joining tree analysis using the Tamura-Nei substitution model with 100 bootstraps was used to illustrate the sequences dissimilarities in *P. destructor* ITS1 region, selected for the assay design, and identify the closest *Peronospora spp*. to be used in the validation assay. Then, the specificity of the primers and probe was assessed with DNA of *Peronospora spp*. closely related to *P. destructor*, other Peronosporales and pathogens isolated from onions.

5.3.4 Internal control and assay sensitivity.

To detect any variation in the ability of the real-time qPCR assay to detect and quantify inoculum due to the presence of PCR inhibitors, an internal control (IC) was multiplexed to the *P. destructor* assay (Fall et al. 2015a; Van der Heyden et al. 2019; Bilodeau et al. 2012). The IC consist of a random DNA sequence added at a constant concentration to the real-time qPCR mixture. Hence, a second set of primers and probe aiming at amplifying the IC (EIPC1MT) was multiplexed with the principal assay (Fall et al. 2015a). To determine the highest concentration of IC to be added to the real-time qPCR mixture without affecting the principal real-time qPCR efficiency. The assessment of the assay sensitivity was conducted with a 5-fold serial dilution of a *P. destructor* sporangia suspension, ranging from 15,625 to 1 sporangium and with a gBlock-based standard curve of the ITS-1 region ranging from 6×10^6 to 6×10^1, with and without the IC. The gBlock consist of a 178bp synthetic DNA fragment (IDT DNA technologies, IA, USA) that includes the region amplified by the primers and probe set and converted into copy number ((concentration of gBlock) / (molar mass) × (6.022 × 1023)) (Carisse et al. 2009). DNA extraction

from sporangia was performed following the protocol described above, except that resuspension buffer contained 5% chlex100 (Bio-Rad Laboratories), UltraPure Salmon Sperm DNA (Thermo Fisher Scientific) at 10 ng μl^{-1} and the EIPC1MT gBlock gene fragment at 2 x 10^2 copies μl^{-1}. The dilution buffer was the same as the extraction buffer without the chelex100 resin. Linear regression analysis was used to describe the relationship and best fit line for *P. destructor* sporangia and amplicon based standard curves with and without the IC and a *t*-test was used to compare the slopes and intercepts of the regression curves obtained.

5.3.5 Real-time qPCR conditions.

The real-time qPCR assay was conducted in a Quantstudio 3 instrument (Thermo Fisher Scientific) with the following cycling conditions: 5 min at 95°C followed by 40 cycles of 30 sec at 95°C, and 30 sec at 62°C. Each reaction consists of 1x ECO master mix (Thermo Fisher Scientific), 250 nM of PdestF and PdestR primers, 150 nM of PdestP Probe, 200 nM of EIPC 100R and EIPC 100F primers, 100 nM of the EIPC probe (Table 2), 0.2 µg/µl of BSA and an additional 6 mM $MgCl_2$. For the soil samples, 600 copies of the IC were also added to the real-time qPCR master mix, unlike the air samples where it was added to the extraction buffer.

5.3.6 Validation of the assay with artificially inoculated samples.

The sensitivity of the designed assay for soil was evaluated by inoculating soil samples with known quantity of *P. destructor* sporangia. These sporangia were collected from infected onions leaves bearing fresh sporulation using a BBL sampling swab (Fisher Scientific). Sporangia were resuspended in sterile distilled water and the suspension concentration was adjusted with an hemocytometer to obtain a concentration of 10,000 spores ml^{-1}. Then, sterilized soil samples, obtained after two heating cycles at 121°C for 20 minutes each, were inoculated with this suspension to obtain concentrations of 5,000, 1,000, 500, 100, 50 and 10 sporangia per gram of dry soil. DNA extraction from soil samples were achieved with the FastDNA spin kit for soil (MP Biomedicals) as described in (Van der Heyden et al. 2019) and quantification of *P. destructor* sporangia was performed with the developed procedure. This experiment was repeated three times. The results of this experiment were used to predicted number of sporangia equivalent per gram of dry soil. To achieve this, the predicted number of sporangia per gram of dry soil was regressed against the predicted value of ITS copy number for Cq values of 16 to 33.

To assess the sensitivity of the designed assay for air samples, *P. destructor* sporangia were deposited on spore sampler rods and counted with a microscope at a 200X magnification. Rods containing 1, 2, 3, 7, 10, 15, 25 and 150 sporangia were submitted to the procedure developed in this study. DNA extraction was performed using the procedure described above, and sporangia counts was performed using the real-time qPCR assay. For this experiment, each concentration had three or four biological replicates and two technical replicates.

5.3.7 Validation of the assay with environmental samples.

A total of 291 soil samples collected from commercial fields located in the Napierville County (Qc, Canada) were collected in the spring from 2017 to 2019. Field selection was based on ODM frequency over the last decade and crop rotation length. Soil samples were collected and prepared following the protocol described in (Van der Heyden et al. 2019). Briefly, soils were sampled according to a random pattern and taken from the first 20 cm from the surface, for a total weight of 250 g per sample. Each sample was air dried at room temperature for 24 to 48h and homogenized using a mill grinder until the obtention of a fine powder. DNA extraction was performed from 200 mg of soil using the method described above.

For airborne samples, a preliminary validation assay was conducted with nine rotating arms impaction spore samplers (Carisse et al. 2009) installed from June 15 to July 13, 2017, in an onion field where ODM was present. The number of *P. destructor* sporangia in each sample was determined under a microscope at a magnification of 250X and with the real-time qPCR procedure developed above.

In 2018-2019, a second validation was done using samples obtained from a network of spore samplers driven by fresh vegetables growers in the Napierville County to estimate the risks associated with *Botrytis squamosa* (Carisse et al. 2012; Carisse et al. 2017; Van der Heyden et al. 2012). In 2018, 16 rotating arms impaction samplers were sampling from May 24 to August 16, for a total of 560 analyzed samples. In 2019, 15 rotating arms impaction samplers were used from June 2 to August 20, for a total of 525 analyzed samples. In 2018 and 2019, the airborne *P. destructor* sporangia concentration was estimated using the real-time qPCR assay developed in this study. The samplers were in operation three times a week from 8:00 to 14:00 h, 50% of the time for a total of 3 897 L of air per sample. To investigate the relationship between airborne inoculum and disease incidence, ODM incidence expressed as percent of onion plants diseased

was assessed weekly in each field where a sampler was operated. In addition, the risk of *P. destructor* sporulation was calculated according to the DOWNCAST predictor (Sutton and Hildebrand 1985).

5.3.8 Statistical analysis.

Linear regression analyses were used to describe the relationship and best fit line for *P. destructor* sporangia and gBlock based standard curves with and without the IC. A one-sided t-test was used to compare the slopes and intercepts of the regression curves obtained. The data obtained in the sensitivity tests for soil and air samples were analyzed using an analysis of variance (ANOVA) followed by an LSD multiple comparison procedure, to determine if the Cq values were different for each of the tested concentrations. All the statistical analyses were performed using SAS/STAT software (version 9.4, SAS Institute Inc. Cary, NC, USA).

5.4 Results

5.4.1 Primers and probe development.

The alignment of 108 sequences (ITS1 region) from 44 species allows for the identification of SNPs specific to *P. destructor* and for the design of specific primers and probe amplifying a 119 bp fragment (Table 1). Neighbor joining tree analysis showed that the region chosen for the development of the assay was conserved amongst *P. destructor* isolates and helped identify *Peronospora* spp. that needed to be included in the validation assay (Figure 1). These results therefore showed that the portion of the ITS1 sequence of *P. cf. ducometii*, *P. cf. fagopyri* and *P. cf. polygoni* had the highest percentage of similarity with *P. destructor*. Moreover, for these three species, the section of ITS1 used for the assay design was identical, facilitating the assessment of specificity, given the limited access to obligate biotrophs DNA. *Peronospora arborescens* and *P. farinosa* isolates also showed a high level of similarity compared with *P. destructor* (Figure 1). For these species, the specificity of the assay was conferred by the presence of SNP on the probe and on the forward primer. Moreover, in order to avoid false positive results, the reaction stringency was increased by optimizing the annealing/extension temperature to 62°C.

5.4.2 Assay specificity, reproducibility, sensitivity and internal control.

As a control, all DNA were tested using the DC6-ITS4 (or ITS1-ITS4) primers set. This step was necessary because DNA extraction from *Peronospora* spp. was carried using a chelex100-

based method and that spectrophotometer-based method of measurement of concentration and purity of DNA obtained with this method is less accurate. The PdestP probe and PdestF/R primers were able to repeatably and reliably amplify *P. destructor* isolates from different sampling years or geographic locations, while no amplification occurred with species showing higher sequence similitudes or with the other fungi species tested (Table 2). All isolates used in the validation assay were amplified with the DC6-ITS4 (or ITS1-ITS4) primer set (Table 2).

Both the sporangia-based and the gBlock-based standard curves displayed a linear dynamic range of amplification, suggesting a high sensitivity of the assay. For the sporangia-based standard curve, the slope of the linear regression was -3.531 and the amplification efficacy was 91.96%. For the gBlock-based standard curve, the slope of the linear regression was -3.525 and the amplification efficacy was 92.17% (Figure 2a). The addition of the IC, optimized at 600 copies, did not affect the amplification efficiency or slope of the linear regression. The slope and amplification efficiency were still -3.528 and 92.06% for the sporangia-based standard curve, and -3.526 and 92.14% for the gBlock-based standard curve (Figure 2b).

5.4.3 Validation of the assay with artificially inoculated samples.

The first validation step consisted of inoculating sterilized chernozem soils with different concentrations of *P. destructor* sporangia (Figure 3). The linear regression conducted with the obtained standard curve showed a negative correlation between the Cq values and *P. destructor* sporangia concentration ($y = -3.08x + 36.32$, $R^2 = 0.98$) and provided a significant fit of the data ($P < 0.0001$). In addition, the results of the analysis of variance followed by a multiple comparison test (LSD) suggested that the Cq values for each concentration were different from each other ($P < 0.0001$). The Cq values ranged from 24.39 Cq, for 5,000 sporangia per gram of dry soil and 32.87 Cq, for 10 sporangia per gram of dry soil (Figure 3). From this superimposition of standard curves, a conversion factor of 0.81 was calculated to obtain sporangia equivalent from ITS1 copy number.

A significant linear relationship between the log transformed sporangia concentration on impaction traps samples rods and Cq values was also observed (Figure 4). The correlation was negative ($y = -3.59x + 31.52$; $R^2 = 0.93$) and provided a significantly good fit of the data ($P < 0.0001$). The Cq values ranged from 31.46 Cq for 1 sporangium to 24.44 Cq for 150 sporangia.

The majority of the mean Cq values were within the 95% confidence interval, except for 2 and 10 sporangia per samples rods (Figure 4).

5.4.4 Validation of the assay with environmental samples.

Among the 290 samples collected from onion grower's fields, concentrations ranged from 0 to 810 sporangia-equivalent per gram of soil. 58 samples (20.0%) were positive for *P. destructor,* 9.0% of the samples were positive but considered to be under the quantification limit. For the other samples, 3.4% had between 10 and 15 sporangia-equivalent per gram of soil and 6.6% had above 15 sporangia-equivalent per gram of dry soil (Figure 5). Soil samples collected from fields where onions have never been grown were all negative for *P. destructor*. The use of sterile soil samples verified that there was no non-specific amplification. However, since no other detection methods were available, type I and type II error could not be assessed. In general, the average soil borne inoculum concentration tend to be correlated with the crop rotation length ($P = 0.053$) (Figure 5).

For airborne samples, validation was first performed by comparing microscopic counts with real-time qPCR counts. During this first step, nine spore samplers were placed in an infected field in 2017 and 58 samples were collected and analyzed. The relationship between microscopic and real-time qPCR counts was linear and the linear regression provided a significantly good fit of the data ($Sporangia_{qPCR} = 1.01 * Sporangia_{\text{microscope}} + -0.99$; $R^2 = 0.96$, $P < 0.0001$). The regression slope was not significantly different from 1 and the intercept was not significantly different from 0 (Figure 6).

In 2018, airborne sporangia concentrations were relatively low with only 24 positive samples out of the 560 tested. The majority of the sporangia were caught between June 24 and July 1 and the highest concentration (4 sporangia m^{-3} of air) was measured on July 1 (Figure 7A). Sporangia have also been caught between July 19 and August 2 and correspond to the first ODM observation date (Figure 7A). In 2018, the disease was only observed in three of the monitored field on August 7, 9 and 16 (Figure 7A). In 2019, airborne sporangia concentration was greater than in 2018 with 42 positive samples out of the 525 tested. Sporangia concentrations were irregular at the beginning of the growing season and increased toward the end of it (Figure 7B). Airborne sporangia concentration varied from 0 to 33 sporangia m^{-3} of air. Sporangia were caught on the 6, 11, 25 and 27 June as well as the 2 and 9 July. The first ODM observation was made on July 10 and incidence increased until August 14 (Figure 7B). In 2018, 11 DC period were observed, six between May 25

and July 1, only one between 1 July and 10 August and four in between 10 and 20 August) (Figure 7A). In 2019, 14 DC period were observed, six between May 25 and July 1, four between 1 July and 10 August and four in between 10 and 20 August) (Figure 7B).

5.5 Discussion

In this study, a real-time qPCR assay was developed for the detection and monitoring of *P. destructor* primary inoculum in soil and secondary inoculum from spore traps samples. Despite the importance of ODM for onion growers in southwestern Quebec and elsewhere, knowledge of its epidemiology remains limited, especially with respect to sources of inoculum. *Peronospora destructor* can develop systemic infections in plant tissues, producing thousands of short-lasting sporangia and several long-lasting oospores (Palti 1989). The sporangia are known to be dispersed over distances ranging from a few meters to several kilometers. Locally they are responsible for the majority of secondary infections and, as the disease spreads, they are also responsible for initial infections in neighboring regions (Aylor 2017). Sources of primary inoculum are believed to be systemically infected onion bulbs, plant parts and soil containing oospores (Palti 1989). Oospores of *P. destructor* are known to remain viable in soil for up to 25 years (McKay 1957), but their role in the epidemiology of the disease remains unclear. In theory, *P. destructor* is not known to survive North American winters even if there is no evidence to confirm or disprove this hypothesis. In a recent study, however, the frequency and intensity of seasonal ODM epidemics in southwestern Quebec were closely correlated to variables associated with the production and survival of overwintering inoculum (Van der Heyden et al. 2020).

The dynamic range of amplification of the developed assay was linear over six orders of magnitude and the real-time qPCR efficiency obtained with the gBlock standard curve and through a dilution of sporangia suspension were 92.06% and 92.14%, respectively. However, using a standard curve based on a dilution of DNA extracted from a suspension of sporangia is a good approach to assess the assay sensitivity and efficiency (Carisse et al. 2009; Fall et al. 2015c), it also important to perform a standard curve that more accurately reflects reality. Hence, the validation conducted with increasing number of sporangia deposited on greased sample rods showed similar linearity and efficiency.

Many studies described the development of real-time qPCR assays for the detection and quantification of oomycetes, especially for *Pythium* spp. and *Phytophthora* spp. (Bilodeau et al.

2009; Cullen et al. 2007; Fall et al. 2015a; Hussain et al. 2005; Kernaghan et al. 2008; Kunjeti et al. 2016; Lees et al. 2019; Li et al. 2014; Li et al. 2010; Lievens et al. 2006; Than et al. 2013; Van der Heyden et al. 2019; Bilodeau et al. 2014; Miles et al. 2017; Schroeder et al. 2006; Spies et al. 2011). However, fewer assays were developed for obligate biotrophic *Peronospora* spp. causing downy mildew diseases (Klosterman et al. 2014; Montes-Borrego et al. 2010; Mota et al. 2011; Belbahri et al. 2005), while the only published assay aiming at detecting *P. destructor* inoculum was based on the use of monoclonal antibodies combined with a lateral flow device (Kennedy and Wakeham 2008). It is generally accepted that action thresholds for downy mildew are relatively low, in the range of 10 sporangia m^{-3} of air (Dhar et al. 2019; Fall et al. 2015a; Fall et al. 2015b). However, the monoclonal antibodies assay was developed for detecting *P. destructor* sporangia from air samples had a limit of detection of 500 sporangia, which limits its practical applications (Kennedy and Wakeham 2008). From this perspective, the present study represents a significant improvement of the approach, since it allows the detection of a single sporangium per impaction trap sample rod.

The sensitivity of the present assay is similar to that obtained in various studies. For example, assays developed for *B. lactucae* (Kunjeti et al. 2016) or *Phytophthora infestans* (Fall et al. 2015a), were also sensitive enough to detect one sporangium per impaction sampler rod. In this study, primers and probe targeting *P. destructor* ITS1 region was used for the development of the assay. The use of high copy number regions such as ITS is essential to achieve low sensitivity (Klosterman et al. 2014; Montes-Borrego et al. 2010; Mota et al. 2011; Belbahri et al. 2005; Gent et al. 2009). The utilization of unique locus in the mtDNA region also showed high sensitivity in addition to high specificity (Bilodeau et al. 2014; Kunjeti et al. 2016; Miles et al. 2017). High throughput sequencing approaches are increasingly being used, including for the development of specific DNA markers and identification of plant pathogen in environmental samples. However, the associated costs are still high, and time required to process the samples is too long for disease management decisions. Given the availability of DNA sequences in public database, the use of directed sequencing approach for the identification of species-specific regions remains an effective and affordable approach for the development of real-time qPCR assays, when possible.

The results obtained in this study suggested that *P. destructor* can be detected in air samples using rotating arms impaction sampler as described in other studies (Dhar et al. 2019; Klosterman et al. 2014; Kunjeti et al. 2016; Carisse et al. 2012; Carisse et al. 2009; Fall et al. 2015c; Van der

Heyden et al. 2012; Van der Heyden et al. 2014). The validation of the assay under commercial conditions was carried out in 2018 and 2019, two different years in regards of regional disease incidence and severity. Airborne *P. destructor* sporangia concentrations showed a high level of seasonal variation. However, in both years *P. destructor* sporangia were caught between 5 and 15 days prior to symptom observations. Similar observations were also made for other oomycetes. In the New Brunswick province, *P. infestans* sporangia were detected six to seven days before the apparition of first potato late blight symptoms (Fall et al. 2015c), while for *B. lactucae* sporangia, airborne inoculum concentrations were used effectively to trigger fungicide applications in lettuce (Dhar et al. 2019). The cumulative risk indices for 2018 were almost 25% less than for 2019. In addition, the risk was mainly distributed at the beginning and end of the season. This could help explain the difference in disease incidence between the two seasons.

Although molecular markers have been developed to detect different *Peronospora* spp. in air and plant samples, few markers are available to investigate their presence and abundance in soil. The assay developed in this study was sensitive enough to detect less than 10 sporangia per gram of dry soil, with a linear dynamic range of amplification from 5,000 to 10 sporangia per gram of dry soil. No such quantitative assay was described in the literature for *Peronospora* spp. in soil, but similar assays were developed for soil borne oomycetes, mainly *Pythium* spp, *Phytophthora* spp. and *Aphanomyces* spp., with sensitivity ranging between 1 and 50 oospores per gram of soil (Almquist et al. 2016; Gangneux et al. 2014; Van der Heyden et al. 2019). In this study, however, sporangia were used instead of oospore due to the difficulty of producing them *in vitro*. However, to confirm that it is possible to detect primary inoculum, soil samples were collected in the spring. Thus, it was assumed that short-lived sporangia or mycelium were absent at that time of year (after a seven months' delay between onion harvest and sampling) and that detected inoculum consisted mainly if not solely of oospores. Soil samples taken from fields with no onion cultivation history were all negative for *P. destructor*. In addition, among the 290 soil samples from onion fields analyzed in this study, almost 20% were positive for *P. destructor* (up to 162 sporangia equivalent per gram of dry soil), supporting to some extent the hypothesis of overwintering under northern regions such as Eastern Canada.

Oospores produced by most *Peronospora* spp. go through a variable period of constitutive dormancy that can last many years (Judelson 2008). This dormant state can be caused by barriers to nutrients entry or auto-inhibitors and as a result, oospores germination in the population does

not take place at the same time, even if environmental conditions are favorable (Judelson 2008). For *P. viciae,* oospores with a maturation period of less than three years were considered to be ineffective (van der Gaag and Frinking 1997c, b), while *P. destructor* oospores needed a maturation period of at least four years (McKay 1957; McKay 1937). For these reasons, the correlation between oospores density and disease incidence has hardly been demonstrated. For example, concentrations between 2 and 21 oospores per gram of soil of *P. viciae* were associated to disease incidence between 17 to 75% (Van Der Gaag and Frinking 1997a). This pathogen's strategy, which aims to prevent the simultaneous germination of all the oospores of a given population, could somehow promote the establishment and maintenance of the disease in a specific area by allowing for the accumulation of inoculum from one cropping season to the next. The results obtained in this study suggest higher soil borne inoculum level in samples collected from fields with shorter rotation length, supporting the hypothesis of inoculum build-up.

Knowledge of both primary and secondary inoculum concentration is essential for tactical and strategic decision-making, and precise monitoring tools are fundamental in achieving accurate decision. Spore sampling coupled with real-time qPCR quantification assays has proven to be efficient in improving tactical decision in onion and other crops (Carisse et al. 2012; Carisse et al. 2017; Dhar et al. 2019; Fall et al. 2015c; Van der Heyden et al. 2012; Klosterman et al. 2014; Kunjeti et al. 2016), while quantification of soil-borne inoculum allows for strategic short-term decisions (Van der Heyden et al. 2019). The assay developed in this study will contribute to the development of integrated disease management strategies and provides a new tool for the monitoring *P. destructor* populations. Several questions certainly continue to arise: could climate change alone be responsible for the increase in the frequency of seasonal epidemics or is this a genetic driven adaptation? With increased trade, are mixed populations capable of surviving our winters? Hence, in addition to better understanding the role of oospores in downy mildew epidemics, it seems essential to improve our knowledge of population genetics of *P. destructor*.

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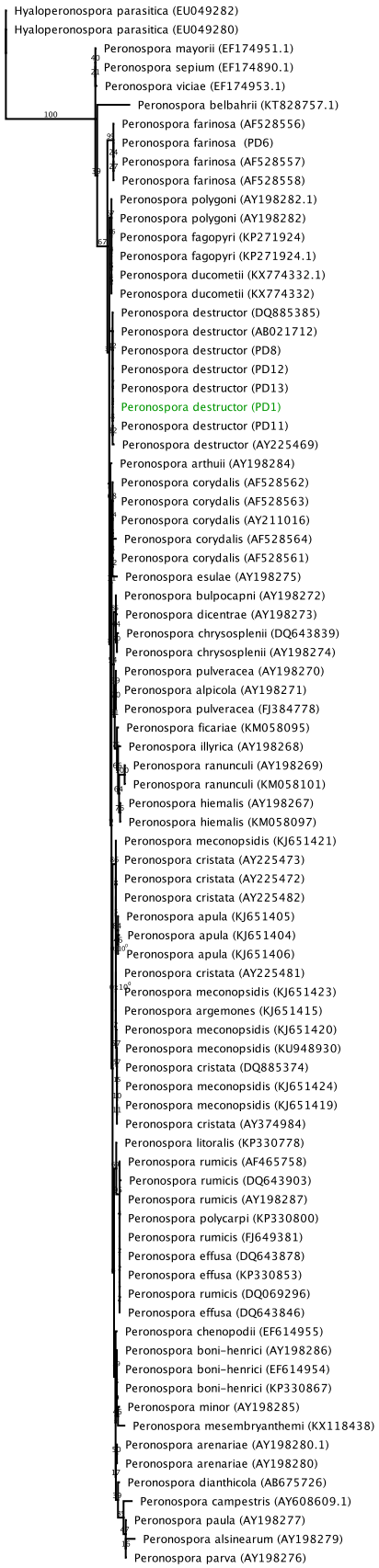

Figure 5-1. One of the most parsimonious trees based on the ITS sequences from 83 *Peronospora* spp. obtained in this study and gathered from the GenBank database.

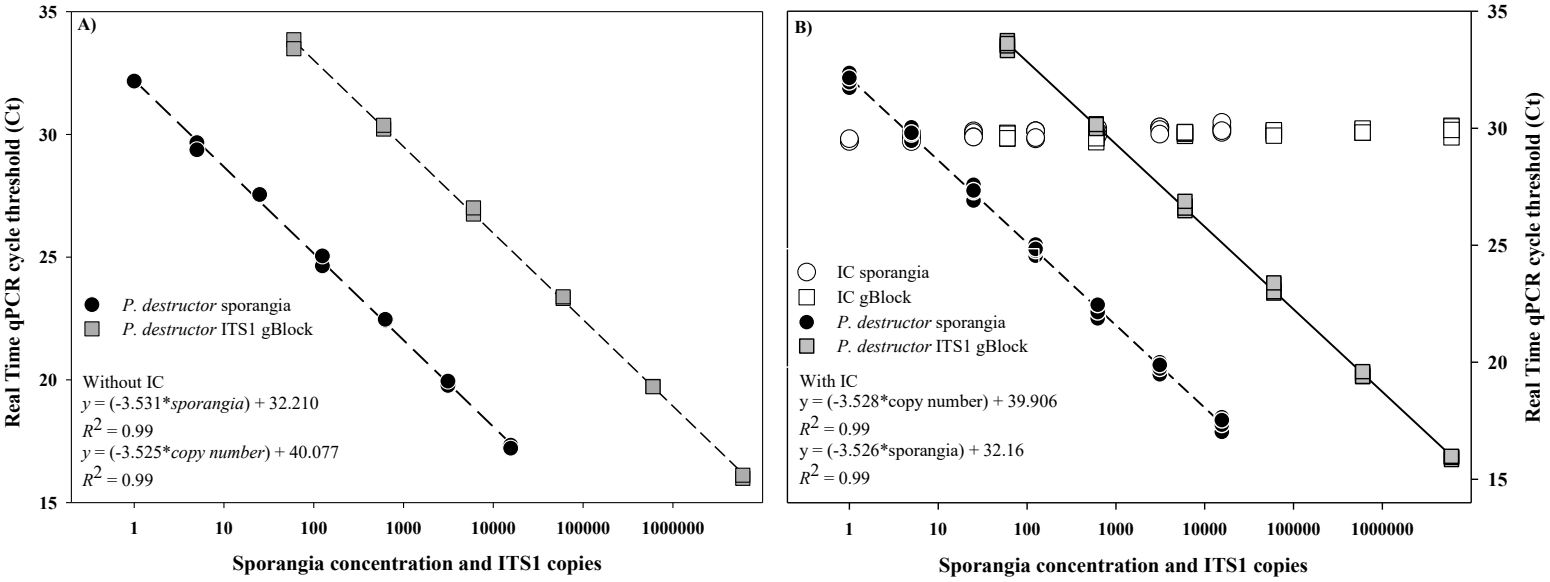

Figure 5-2. Sporangia- and gBlock-based standard curves of *P. destructor* qPCR quantification threshold (Cq) values of *P. destructor* ITS2 were plotted against the log10 starting quantity. A) without and B) with the addition of the internal control. The results were obtained from five independent real-time qPCR runs. The black circles a represents amplification of the sporangia-based standard curves while the grey squares represent amplification of the gBlock based standard curves with the addition of the internal control.

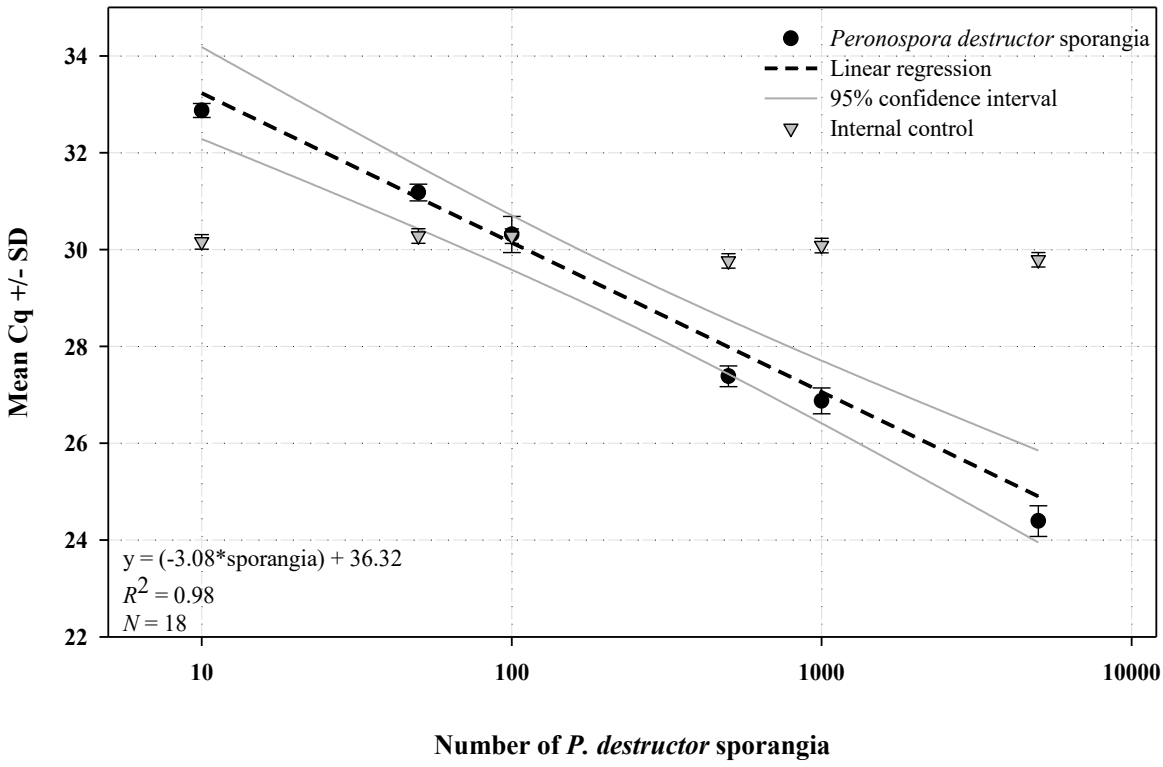

Figure 5-3. Standard curves of *P. destructor* sporangia concentration obtained in the validation assay in soil, with quantification threshold (Cq) value plotted against the $\log_{10}$ starting quantity. For this validation assay, *P. destructor* sporangia were artificially inoculated in sterilized soils at

concentrations ranging from 5000 to 10 sporangia g^{-1} of dry soil

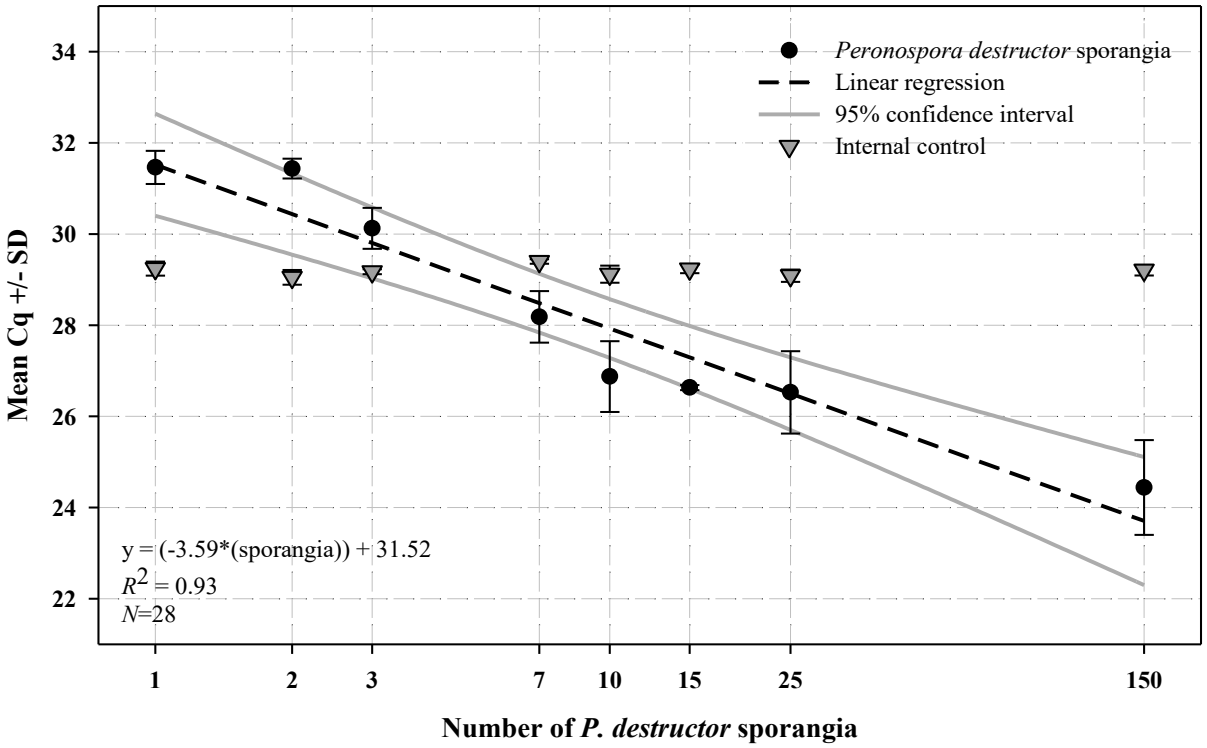

Figure 5-4. Results of the first validation assay during which sporangia artificially deposited on plastic rodsand processed using the qPCR assay developed in this study. The eight points standard curve is based on the amplification of purified DNA from 1 to 150 sporangial count. Each point represents the average and standard deviation of 3 or 4 biological replicates. The linear regression was conducted using all the points.

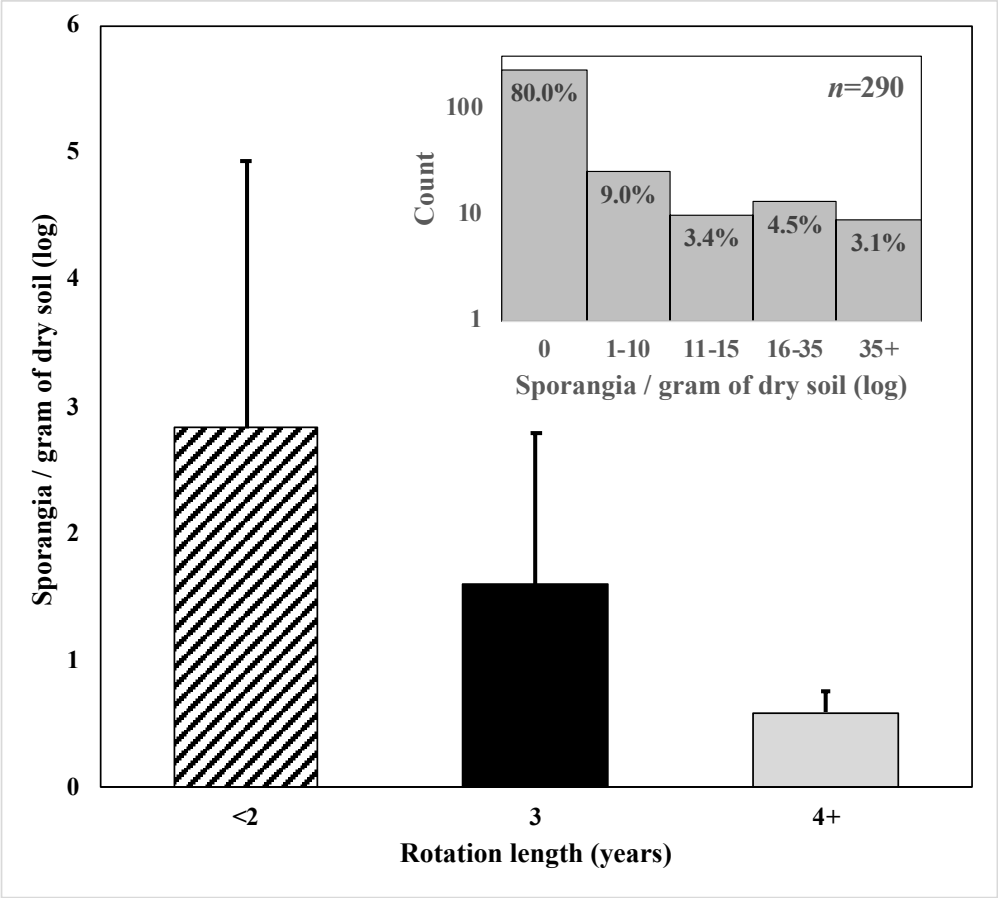

Figure 5-5. Field validation of the real-time qPCR assay for soil samples. The embedded histogram represents the frequency distribution of the 291 soil samples collected and tested in this study. The results are also presented according to a classification based on rotation length. The dashed bar represents the average soil borne sporangia concentration for a rotation length of two years or less, the black bar represents a rotation length of three years and the grey bar a rotation length of four years or more. The error bar represents the standard deviation for the category.

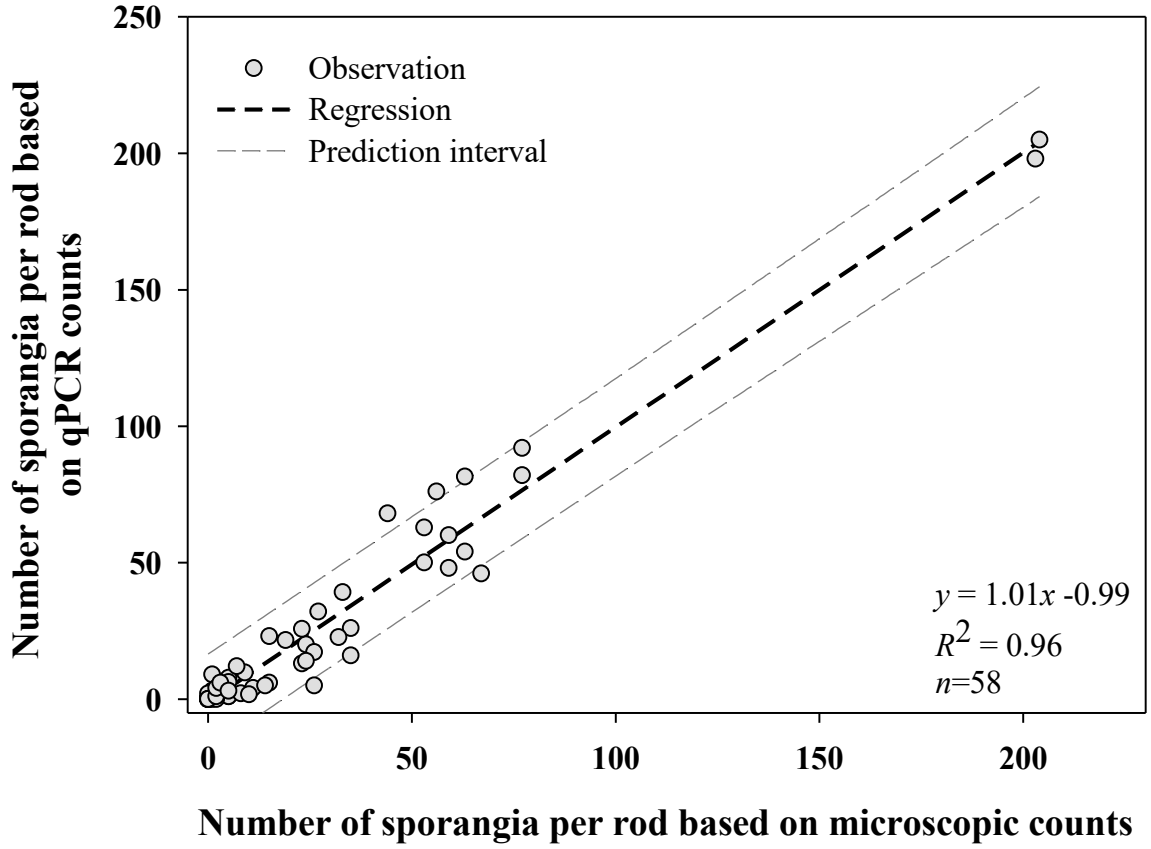

Figure 5-6. Field validation of the qPCR assay.Aerial samples were collected in 2017 from an infected onion field, using nine impaction samplers. Sporangia enumeration was conducted with the qPCR assay and compared with microscope counts. The figure shows linear regression between the number of P. destructor sporangia obtained from microscope counts compared with the estimation obtained with the qPCR assay.

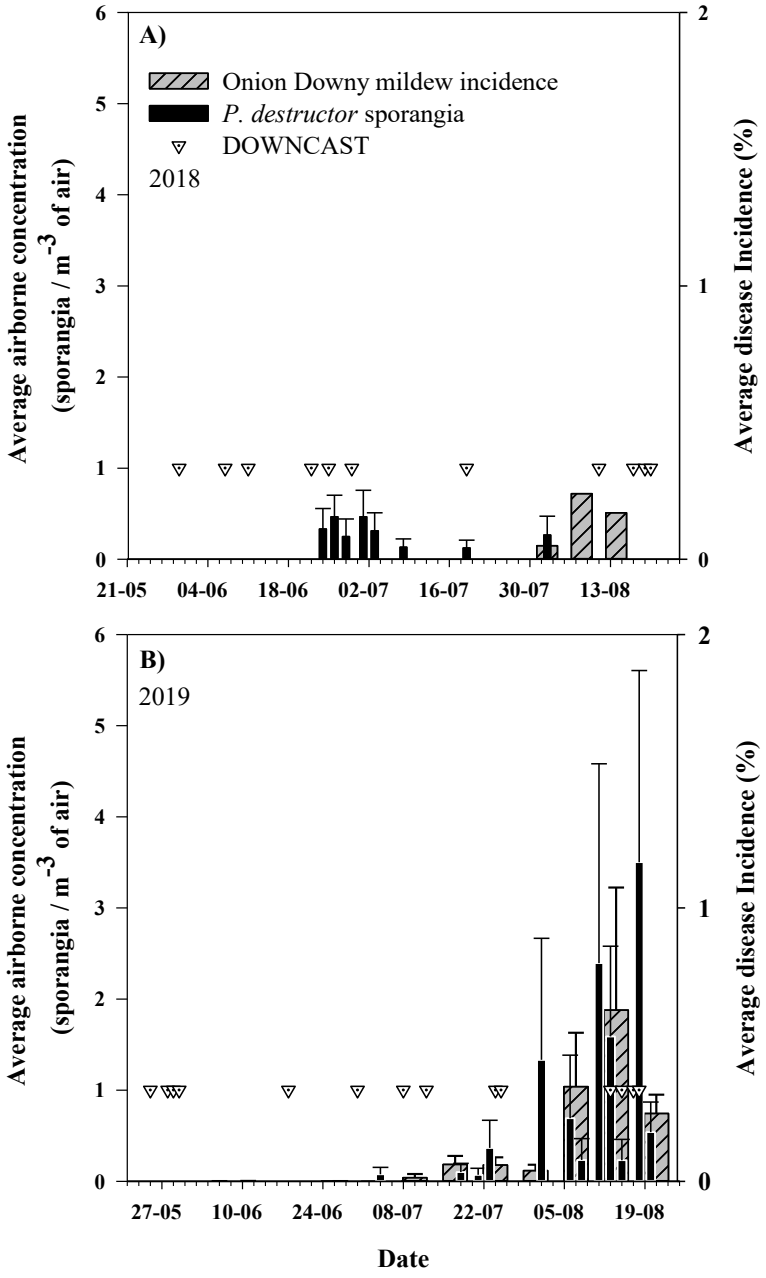

Figure 5-7. Field validation of the qPCR assay conducted in commercial fields in A) 2018 and B) 2019 using a network of impaction traps. In both panels, the black dot represents the average airborne *P. destructor* concentration, and the error bars represent the standard deviation. The grey bars represent the average onion downy mildew incidence, and the error bars represent the standard deviation. In both panels, the triangles indicate the risk of downy mildew sporulation according to DOWNCAST.

Table 5-1. Isolates used to test the specificity of the designed assay.

ID	Sampler[a]	Specie	Host	Origin	DC6/ITS4[b]	Pdest-F/R Pdest-P[b]
Qc1	1	*Peronosora destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc2	1	*P. destructor*	Dry bulb onion	Sainte-Clothilde, Canada	+	+
Qc3	1	*P. destructor*	Bunching onion	Sainte-Clothilde, Canada	+	+
Qc4	1	*P. destructor*	Bunching onion	Hemingford, Canada	+	+
Qc5	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc6	1	*P. destructor*	Bunching onion	Sainte-Clothilde, Canada	+	+
Qc7	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc8	1	*P. destructor*	Bunching onion	Sainte-Clothilde, Canada	+	+
Qc9	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc10	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc11	1	*P. destructor*	Dry bulb onion	Sainte-Clothilde, Canada	+	+
Qc12	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc13	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc14	1	*P. destructor*	Dry bulb onion	Napierville, Canada	+	+
Qc15	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
Qc16	1	*P. destructor*	Bunching onion	Sainte-Clothilde, Canada	+	+
Qc17	1	*P. destructor*	Dry bulb onion	Sherrington, Canada	+	+
FR1	2	*P. destructor*	Shallots	Bretagne, France	+	+
FR2	2	*P. destructor*	Shallots	Bretagne, France	+	+
CA1	3	*P. destructor*	Dry bulb onion	California, USA	+	+
ON1	5	*P. destructor*	Dry bulb onion	Ontario, Canada	+	+
	4	*Peronospora ducometi*	Buck wheat	Washington	+	-
	1	*Peronospora farinosa*	Chenopodium	Napierville, Canada	+	-
	1	*Peronospora effusa*	Spinach	Napierville, Canada	-	-
	1	*Peronospora belbahrii*	Basilic	Ste-Hyacinthe, Canada	+	-
	2	*Peronospora belbahrii*	Basilic	Bretagne, France	+	-
	1	*Peronospora arborescens*	Poppy	Ste-Hyacinthe, Canada	+	-
	1	*Peronospora lamii*	Salvia	Ste-Hyacinthe, Canada	+	-
	1	*Pseudoperonospora cubensis*	Cucumber	Leamington, Canada	-	-
	2	*Peronospora viciae*	Pea	Bretagne, France	+	-
	2	*Hyaloperonospora parasitica*	Radish	Bretagne, France	+	-

1	*Plasmopara viticola*	Grape	Napierville, Canada	+	-
1	*Bremia lactucae*	Lettuce	Napierville, Canada	+	-
1	*Phytophthora infestans*	Potato	Napierville, Canada	+	-
1	*Phytophthora capcici*	Cucurbit	Joliette, Canada	+	-
1	*Aphanomyces euteiches*	Pea	Trois-Rivières, Canada	+	-
1	*Botrytis cinerea*	Strawberry	Ste-Hyacinthe, Canada	+	-
1	*Botrytis squamosa*	Onion	Napierville, Canada	+	-
1	*Stemphylium versicarium*	Onion	Napierville, Canada	+	-
2	*Fusarium oxysporum*	Onion	Bretagne, France	+	-

[a] Isolates were obtained from: 1: This study; 2: Dr. Celine Hamon, Vegenov, St-Pol de Leon, Bretagne, France; 3: Dr. Alexander Putman, University of California, Riverside, CA, USA; 4: Dr. Lindsay du Toit, Washington State University, Mount Vernon, WA, USA and 5: Mr. Travis Cranmer, OMAFRA, Ontario Canada.

[b] DC6/ITS4 and Pdest-F/R Pdest_P are primers/probe combination.

Table 5-2. Description of the primers and probes used in this study.

Primer/probes name [a]	Nucleotide sequence	Reference
DC6	5'-GAGGGACTTTTGGGTAATCA -3'	Cooke et al. (2000)
ITS-4	5'-TCCTCCGCTTATTGATATGC-3'	White et al. (1990)
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'	
PdestF	5'-CCAACCGAGGTCAGAACACTCG-3'	This study
PdestP (5'-FAM)	5'-CAAAAACATGCCACCAGCAGCCGCCAAGTAA-3'	
PdestR	5'-CGTGAACCGTATCAACCCAATTAA-3'	
EIPC100F	5'-AGGCTAGCTAGGACCGATCAATAGG-3'	Fall et al. (2015)
EIPC100P (5'-HEX)	5'-CCTATGCGTTCCGAGGTGACGACCTTGCC-3'	
EIPC100R	5'-AGTGCTTCGTTACGAAAGTGACCTTA-3'	

[a] Each probe was quenched with QSY at the 3' end.

Connecting statement for chapter 6

Chapters 4 and 5 reported evidence that at least part of the inoculum is local. The dispersal patterns, inoculum sources, and reproductive mode of organisms greatly influence the genetic diversity of their populations. Thus, evolutionary processes and demography can be inferred from population structure, which is defined by patterns of genetic diversity within and between populations. Therefore, in Chapter 6, genotyping by sequencing was used to describe the genetic structure of *P. destructor* populations in southern Quebec. A genetic structure characteristic of clonal organisms, a significant effect of region on genetic variation, and patterns of isolation by distance were identified. In other words, these results suggest that diversity in southern Quebec is essentially due to local mutations leading to a slow divergence of populations in time and space. Furthermore, these results reveal for the first time a high level of heterozygosity indicating heterothallic reproduction.

This Chapter was submitted and is now published in Molecular Plant Pathology, a journal published jointly with the British Society for Plant Pathology. This journal publishes articles in molecular plant pathology that advance our knowledge of the molecular basis of diseases or their management, including research on diseases caused by oomycetes, molecular analyses of pathogens, pathogen evolution, or the interaction of pathogens, the environment, and host plants. This manuscript is now published as:

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6 *Genotyping by sequencing suggests overwintering of Peronospora destructor in southwestern Quebec, Canada*

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6.1 Abstract

Several *Peronospora* species are carried by wind over short and long distances, from warmer climates where they survive on living plants to cooler climates. In eastern Canada, this annual flow of sporangia was thought to be the main source of *P. destructor* responsible for onion downy mildew. However, the results of a recent study showed that increasing frequency of onion downy mildew epidemics in eastern Canada is associated with warmer autumns, milder winters, and previous year disease severity, suggesting overwintering of the inoculum in an area where the pathogen is not known to be endogenous. In this study, genotyping by sequencing was used to investigate the population structure of *P. destructor* at the landscape scale. The study focused on a particular region of southwestern Quebec -Les Jardins de Napierville- to determine if the populations were clonal and regionally differentiated. The data was characterized by a high level of linkage disequilibrium, characteristic of clonal organisms. Consequently, the null hypothesis of random mating was rejected when tested on predefined or non-predefined populations, indicating that linkage disequilibrium was not a function of population structure and suggesting a mixed reproduction mode. Discriminant analysis of principal components performed with predefined population assignment allowed grouping *P. destructor* isolates by geographical regions, while AMOVA confirmed that this genetic differentiation was significant at the regional level. Without using a priori population assignment, isolates were clustered into four genetic clusters. These results represent a baseline estimate of the genetic diversity and population structure of *P. destructor*.

6.2 Introduction

Population genetics is essential to understand how ecological and evolutionary processes influence plant pathogen's temporal and spatial genetic diversity (Grünwald et al. 2017; Milgroom 2017). This genetic diversity in plant pathogen populations results from migration, random genetic drift, mutation, recombination, or natural selection (Milgroom 2017). It is generally characterized by the presence of polymorphisms that can be identified through genetic markers, the most commonly used being microsatellites and single-nucleotide polymorphisms (SNPs). Some markers, such as microsatellites and single sequence repeats (SSRs), are well suited to study model organisms (i.e., a well-studied organism for which many tools and resources exist) because they require prior genetic knowledge. Others, such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), allow the amplification of DNA fragments of unknown sequence (Milgroom 2017; Russell et al. 2017). Nevertheless, in both cases, the approach does not allow for deep genomic coverage, potentially limiting the study of organisms with no or limited prior genetic knowledge. Recent developments in high-throughput DNA sequencing tools such as genotyping by sequencing (GBS) (Elshire et al. 2011) allow for simultaneous exploration of genome-wide genetic variation over hundreds of loci in a large number of individuals or populations. Hence, these tools enable a finer understanding of the relationships between genetic, temporal, and geographic variations for non-model organisms (Narum et al. 2013). Moreover, the availability of R codes (The Comprehensive R Archive Network) and resources (Jombart 2008; Jombart et al. 2010; Kamvar et al. 2014; Knaus and Grünwald 2017; Paradis et al. 2017) facilitate the use of these approaches for non-model organisms with a predominantly asexual mode of reproduction.

Many crop pathogens are members of the Peronosporaceae family (Crandall et al. 2017). In this family, particular attention has been given to members of the genus Phytophthora, especially *P. infestans* (Danies et al. 2015; Goss et al. 2014; Hansen et al. 2016; Knapova and Gisi 2002; Montarry et al. 2010; Montes et al. 2016; Sjöholm et al. 2013; Widmark et al. 2007), *P. sojae* (Cai et al. 2019; Gally et al. 2007; Stewart et al. 2015; Stewart et al. 2013; Wu et al. 2017) and *P. ramorum* (Gagnon et al. 2016; Goss et al. 2009; Ivors et al. 2006; Mascheretti et al. 2008; Prospero et al. 2007; Prospero et al. 2004; Vercauteren et al. 2010). Microsatellites and GBS were used to provide more accurate estimates of evolutionary processes by identifying patterns in genetic

structures, describing spatial and temporal patterns in pathogen populations and clonal dynamics, inferring the role of sexual recombination, and identifying the origin of local populations. For example, GBS data revealed smaller genetic clusters within some of the dominant *P. infestans* clonal lineages. In addition to being sourced from neighboring regions through long-distance dispersal, these results suggest overwintering within regions of the United States where it was not expected (Hansen et al. 2016).

Despite their importance, with more than 700 species, obligate biotrophic downy mildews received less attention in population genetics research than other groups of pathogens. The adaptations that led to their biotrophic lifestyle have compromised the efficiency of metabolic pathways necessary for saprophytic growth and thus altered their ability to grow on culture media (Thines and Choi 2015). Hence, the inability of maintaining these organisms on artificial media limited their study. Typically, downy mildews produce numerous wind-dispersed sporangia (asexual spores), spreading the inoculum over short or long distances during the growing season. It is generally accepted that downy mildew pathogen populations are mostly clonal (Bhattarai et al. 2020; Blanco-Meneses et al. 2018; Gent et al. 2019; Lyon et al. 2016; Summers et al. 2015b). However, they are also capable of genetic recombination through sexual reproduction (homothallism and heterothallism), producing oospores, which are long-term survival structures that can, like *P. destructor*, survive up to 25 years in soils (McKay 1957). Hence, given their abundant production of sporangia, their short infection-sporulation cycles, and their high host plant specificity, downy mildew pathogens are particularly destructive and of concern for several crops of economic importance, notably *Plasmopara viticola* in grapevine (Carisse et al. 2020), *Bremia lactucae* in lettuce (Dhar et al. 2019; Fall et al. 2015a), *Pseudoperonospora cubensis* in cucurbits (Granke et al. 2014; Granke and Hausbeck 2011; Summers et al. 2015a), *Peronospora effusa* in spinach (Klosterman et al. 2014), and *Peronospora destructor* in onion (Van der Heyden et al. 2020b; Van der Heyden et al. 2020a; Fujiwara et al. 2021).

Variations between isolates of a given downy mildew species, with respect to pathogenicity or aggressiveness on specific varieties of their host, are commonly observed. For some species, genetic diversity has led to the description of races or pathotypes (Crandall et al. 2017). This is the case for *P. effusa,* for which at least 14 races related to aggressiveness on given spinach cultivars were identified (Feng et al. 2014). *Pseudoperonospora cubensis* is another example where population markers have been associated with mating types or clades, which were associated with

host specificity (Cohen and Rubin 2012; Wallace et al. 2020). In other cases, such as *Pseudoperonospora Humuli,* population markers suggested genetic differentiation in populations at a very small scale; hop yard (Gent et al. 2019). In addition to helping refine the taxonomy of these pathogens, the knowledge of population genetics is useful to improve biosurveillance (Rahman et al. 2020), and ultimately, cultural practices and control of downy mildew diseases. However, as for many of the downy mildews, this type of genetic information is not yet available for *P. destructor*, the causal agent of onion downy mildew (ODM).

ODM has been reported worldwide in almost all the regions where onions are grown (Thines and Choi 2015). It is particularly damaging in cool and humid climates, making it one of the main threats to onion production in Northern Europe and North America. In Canada, onion is an important vegetable with a farm gate value of >95 M CAD$ (Mailvaganam 2017). The production is largely concentrated in the provinces of Quebec and Ontario. In Quebec, onion is produced in basins of muck soils (chernozem) located about 50 km southwest of Montreal, the most important ones being those of Sherrington (SHERR), Napierville (NAP), and Ste-Clothilde (CLO). In these areas, initial inoculum sources are unknown, and *P. destructor* is generally considered a periodically introduced onion pathogen. However, during the last decade and after a 10-year ODM-free period, ODM epidemics have been more frequent, started earlier, and were more severe than in the previous two decades (Van der Heyden et al. 2020b). In a recent study, the polyetic component of ODM epidemiology was shown to be mostly influenced by the precipitation regime, the regional disease incidence towards the end of the previous year, warmer temperatures in the previous autumn, and warmer winters (Van der Heyden et al. 2020b). These results support the hypothesis that *P. destructor* may be overwintering locally, although the authors could not, from the data available, determine whether sexual reproduction was involved or not. Potential sources of inoculum are systemically infected plants (onion sets, volunteer plants, or waste piles), airborne sporangia, and oospores present in soil or plant debris (Hildebrand and Sutton 1980; McKay 1957; Palti 1989). Analysis of soil samples with a real-time qPCR assay suggested the presence of quantifiable DNA in almost 11% of the soil samples analyzed, especially samples taken from fields with a short crop rotation (Van der Heyden et al. 2020a; Fujiwara et al. 2021). In addition, these results suggested local overwintering but did not provide information about population structures.

Given the importance of onion downy mildew and the absence of knowledge on *P. destructor* population diversity, there was a need to explore the genetic variations of *P. destructor*

populations and to provide evidence of local overwintering. In this study, genotyping by sequencing was used to investigate the population structure of *P. destructor* at the landscape scale. Thus, the objective of this research was to estimate the level of genetic diversity and differentiation between and within populations. The study focused on a particular region of southwestern Quebec (Canada) -Les Jardins de Napierville- where isolates were collected between 2016 and 2019 and analyzed together with Californian isolates as an outgroup to determine if the populations were clonal and regionally differentiated. Here, we hypothesize that although *P. destructor* populations are predominantly clonal, these will be differentiated at the scale of the production basins if *P. destructor* survives the winter locally. A fine resolution such as that obtained by GBS will allow the identification of mutations that accumulate within clonal populations as they diverge in space. The results presented in this study represent a baseline estimate of the genetic diversity and population structure of *P. destructor* and will improve our understanding of its epidemiology.

6.3 Material and methods

6.3.1 Population sampling.

The Jardins de Napierville county has about 10,000 ha of cultivated muck soil, of which around 2,000 ha are dedicated to the cultivation of Allium each year (mainly dry bulb onions, French shallots, and green onions). The muck soils are distributed in several basins, the three most important being Sherrington (SHERR), Napierville (NAP), and Ste-Clothilde (CLO). Hence, the populations are defined based on the geographic location of the muck soil basin in which they were sampled.

Peronospora destructor isolates were collected from diseased onion leaves bearing fresh sporangia from 2016 to 2019. Sampling was performed randomly across the three muck soil basins in 41 fields belonging to 15 farms when agronomists and crop specialists reported symptoms. In 2019, a targeted sampling was conducted in three heavily infected fields, from which 10 to 12 isolates were collected on the same sampling date. In addition to the isolates collected in Quebec as described, three isolates were obtained from Hartford County, California. For all isolates, sporangia were taken from single lesions by carefully touching them with a sterile BBL culture swab (Fisher Scientifc, Mississauga, ON, Canada) and stored in 95% ethanol at -20°C.

6.3.2 DNA preparation and genotyping by sequencing.

As the number of sporangia was limited in many isolates, a multiple displacement amplification-based whole genome amplification method (WGA) was used to increase the genomic DNA for all isolates (Casso et al. 2019; Han et al. 2012). To do this, the Repli-g single cell kit was used according to the manufacturer's instruction (Qiagen, Mississauga, ON, Canada). After the WGA procedure, the presence of *P. destructor* DNA was tested using qPCR as described in Van der Heyden et al. (2020a) and then quantified using a Qubit fluorometer with the dsDNA Qubit HS test kit (ThermoFisher, Mississauga, ON, Canada). DNA concentration was adjusted to 10 ng/µl before being sent to the Genomic Analysis Platform of the Institute of Integrative Biology and Systems (IBIS) for GBS sequencing workflow (Elshire et al. 2011). Briefly, DNA was digested with *ape*K1 for genome complexity reduction prior to adapter ligation. All libraries were sequenced on Ion Torrent P1V3 chips, targeting 60 to 80 million single-end reads per chip. Each chip contains 58 isolates, and two rounds of sequencing (two separate chips) were conducted for each library for a total of four chips.

6.3.3 Data processing, variant calling, and quality control.

Demultiplexing was performed using process_radtag from Stacks v2.53 (Catchen et al. 2013; Catchen et al. 2011) and barcodes and sequencing adapter were trimmed using BBDuk v38.86 from the BBTools suite (Bushnell 2014). Reads were mapped to a draft genome of *P. destructor* (GenBank accession no. WBRY00000000 (Natesan et al. 2020) using Bowtie2 v2.4.1 with the "very-sensitive-local" option (Langmead and Salzberg 2012). Identification of SNPs in the metapopulation for each locus was performed using gstack (Stacks v2.53). The program provided a first catalog containing the consensus sequence for each locus and a second one containing genotyping data. These two files were then read by the population program (Stacks v2.53) to generate a Variant Calling Format (VCF) file. To be processed, a locus had to be present in at least three populations and at least in 50% of all individuals.

Single nucleotide polymorphisms were further filtered for quality using the R package *vcf*R (Knaus and Grünwald 2017). First, violin plots were created to inspect the distribution of sequencing depth for variants from each isolate. Isolates with average sequencing depth lower than four were removed from downstream analysis, and a 95% confidence interval of the depth

distribution was created to remove variants with unusually high sequencing depth. Variants with more than 15% and isolates with more than 55% missing data were also removed from the dataset. A Manhattan plot based on Hedrick's G'_{ST} statistics was built using the genetic distance function available in *vcf*R to visualize the distribution of the variant's differentiation among the filtered datasets. For each isolate, heterozygosity was explored by plotting a histogram showing the frequency distribution of allele balance for the first and second most abundant allele, as described by Knaus et al. (2020). The VCF data was converted into format (.bed, .fam, and .bim files) readable by the software PLINK v1.9 (Purcell et al. 2007) and linkage disequilibrium (LD) was calculated as r^2 between SNPs for each contig and 10 Mb windows. The VCF data was further filtered to remove SNPs in perfect LD (LD pruning) using an in-house python script. Finally, uninformative loci were removed using the informloci() function implemented in *poppr* with a cut-off value of 2% and minor allele frequency of 0.01 (Kamvar et al. 2014; Kamvar et al. 2015).

6.3.4 Genetic differentiation and population structure of P. destructor populations.

The data were organized into four datasets. First, the "complete dataset" includes all the isolates retained after the filtration steps. The second one, the "Quebec dataset", was obtained by removing the Californian isolates from the complete dataset. The third data set, called the "regional data set", corresponds to isolates obtained from random sampling in the three muck soil basins, when agronomists and crop specialists reported symptoms. Finally, the fourth dataset, referred to as the "targeted dataset," corresponds to isolates obtained from 2019 sampling in the three severely infested fields. It must be noted that the isolates in the regional and targeted datasets do not overlap.

To determine the genetic structure among *P. destructor* populations, the analysis of molecular variance (AMOVA) method was applied using poppr.amova() from the R package *ade4* (Dray and Dufour 2007). AMOVAs were performed separately with the regional dataset and with the targeted dataset, with significance assessed by performing 999 permutations for each AMOVA. In addition, for both datasets, pairwise AMOVA was performed to calculate the pairwise index of population differentiation Φ_{PT}. These analyses were conducted with the clone-corrected and non-clone-corrected data, with similar results for both; therefore, only the non-corrected data were retained (data not shown).

Discriminant analysis of principal components (DAPC; (Jombart et al. 2010)) was performed on the complete dataset and the Quebec dataset. Because there is a risk of missing useful information or overfitting the data if too few or too many principal components (PCs) are retained, the number of PCs was chosen using function xvalDAPC() from the R package *adegenet* (Jombart et al. 2010). Cross-validation was performed with a training set corresponding to 90% of the data and a validation data set made of the remaining 10%. The procedure was repeated 1000 times at each step of the PC retention. The retained number of PCs should provide the highest mean of success (HMS) and the lowest root mean square error (RMSE), with greater importance given to the lowest RMSE. A dendrogram based on the underweight paired group method with arithmetic mean (UPGMA) was built in *poppr* by grouping populations using Nei's genetic distance.

To test for the underlying population structure, *k*-means clustering was performed with find.clusters() from the R package *adegenet* (Jombart 2008) to group isolates in clusters ranging from 1 to 10 clusters. The value of *k* that returned the most parsimonious fit based on the Bayesian information criterion (BIC) corresponded to the so-called theoretical number of genetic clusters. DAPC was then used with no a priori to group isolates into genetic clusters. A UPGMA dendrogram was built by grouping individual isolates based on Provesti's genetic distance in poppr.

The standardized index of association $\bar{r}_d$ was computed to estimate the degree of linkage within each population defined a priori or not, using the R package *poppr* (Kamvar et al. 2014; Kamvar et al. 2015). To test the null hypothesis of random mating, the statistical assessment of $\bar{r}_d$ estimates was performed with 999 permutations; the rejection of this hypothesis is generally considered to be associated with an asexual mode of reproduction (Agapow and Burt 2001).

The Mantel test was used with the regional and targeted datasets to analyze the relationship between genetic divergence and geographic distance (Mantel 1967). For each of the two Mantel tests, two matrices were created: a matrix of genetic distances calculated with dist.diss() in *poppr* (Kamvar et al. 2014) and a matrix of geographic distances obtained with dist() in *ade4* (Dray and Dufour 2007). The Mantel tests themselves were performed with function mantel.randtest() available in *ade4*, with *P*-values computed from 9,999 permutations (Dray and Dufour 2007). A Mantel correlogram was produced for the Quebec dataset to test for spatial autocorrelation between pairs of isolates at seven distance classes ranging from 1,500 to 15,000 m. The analysis was

conducted as described in (Legendre and Legendre 2012), using function mantel.correlog() available in the R package *vegan* (Oksanen et al. 2016). For each Mantel correlogram ordinate, the Pearson correlation coefficient was computed and tested for significance with 9,999 permutations.

6.3.5 Data availability.

Demultiplexed raw reads obtained for the isolates used in in this project were deposited in the NCBI Sequence Read Archive (SRA) under the accession number PRJNA741398.

6.4 Results

6.4.1 Genotyping and variant calling.

The Ion Proton protocol used at the Institute of integrative biology and systems (IBIS) provided an average of 32.5M reads per sequencing chip, for a total of 52.3M and 77.9M reads for the first and second run, respectively (Supplementary figure 6-1). The difference in the number of reads between the two runs was largely attributable to the first chip, which produced only 15M reads, as opposed to the other three, which produced just under 40M reads each. After demultiplexing, four isolates with low or no reads were dropped, and 50.9M and 76.1M reads were retained for the first and second run. Ultimately, the average number of reads per isolate was 0.5M (Supplementary Figure 6-2A). After alignment to the reference genome (Natesan et al. 2020), an average of 0.24M reads per isolate was kept, and the mean sequencing depth per isolate was 6.75x (Supplementary figure 6-2B-C).

6.4.2 Quality control and filtering.

Prior to quality filtering, genotyping of the remaining 112 isolates yielded a total of 45,792 raw variants in a data set containing 21% missing data. Violin plots showing the distribution of sequencing depth among the variants for each isolate suggest a unimodal distribution, with data generally clustered around a median of 10x sequencing depth (Supplementary figure 6-3). After the filtering steps, an additional nine isolates that did not meet the quality criteria were dropped, resulting in a data set containing 103 isolates (Table 6-1), 5,335 variants, only 2.6% missing data, and a mean sequencing depth per isolate of 17.3x (standard deviation 8.99). The data showed important levels of heterozygosity indicated by a clear peak close to 50% allelic frequency (Supplementary figure 6-4). Linkage disequilibrium analysis suggested that a large proportion of

the SNPs had a non-random association and were strongly linked (Figure 6-1A). This linkage did not appear to decay with physical distance (Figure 6-1B). After removing all SNPs in perfect linkage disequilibrium, 1,340 variants were retained. At this step, there was no evidence that missing data were associated with a given population, that variant loci were missing across isolates, or that given isolates had too much missing data (Supplementary figure 6-5).

Manhattan plots were used to represent the distribution of the G'_{ST} statistic as a function of genomic position for the complete dataset and the targeted subset. These plots showed that most variants had G'_{ST} values near zero, both for the complete data set and the targeted subset (Figure 6-2). However, among all the variants obtained, some were also found to be highly differentiated. In the end, 158 and 168 informative loci were kept for use in subsequent analyses for the complete and the targeted dataset.

6.4.3 Genetic differentiation of P. destructor populations.

The AMOVA performed with the regional dataset revealed that the populations were significantly differentiated at the regional level (P = 0.002), with 17.93% of the genetic variance observed between regions (Table 6-2). Similarly, for the targeted dataset, a significant (P = 0.005) share of the genetic variance (23.98%) was observed between fields (Table 6-3). The result of the AMOVA revealed a significant but limited effect of year on population structure (P=0.041), with 8.47% of the variation observed among years (Supplementary table 6-1). The AMOVA performed with the crop as classification factor to define populations did not show significant differentiation among crops (P=0.581) (Supplementary table 6-2).

The pairwise AMOVAs for the regional dataset suggested a significant level of genetic differentiation among local populations. The largest genetic differentiation was found between the NAP and SHERR populations (Φ_{PT}=0.284, P=0.006), whereas the genetic differentiation between the SHERR and CLO populations, though smaller, was also significant (Φ_{PT}=0.153, P=0.004). In contrast, genetic differentiation between the CLO and NAP populations was not significant (Φ_{PT}=0.006, P=0.292) (Table 6-4). Similar pairwise AMOVA results were obtained for the targeted and Quebec datasets, with the same interpretation in terms of significant or non-significant genetic differentiation between local populations (Table 6-4). When the regional dataset was split into 2019 and 2016-2018, the pairwise AMOVA results showed that the NAP and SHERR

populations were significantly differentiated regardless of the grouping, while the NAP and CLO populations were not differentiated. The SHERR and CLO populations were significantly differentiated for the target group in 2019 but not in 2016-2018 (Table 6-4).

6.4.4 Genetic structure of P. destructor populations.

In the discriminant analysis of principal components (DAPC) with a priori population assignment, 19 principal components (PCs) were retained for the complete dataset (highest mean of success [HMS]=0.660, root mean square error [RMSE]=0.345). This result indicated that isolates obtained from California are well differentiated from all the isolates collected from the three muck soil basins in southwestern Quebec (Figure 6-3A). For the Quebec dataset, 25 PCs were retained in the DAPC (HMS=0.635, RMSE=0.402) (Figure 6-3C). For this dataset, 76.9% of the isolates were correctly assigned to the populations defined a priori. More specifically, 85.4%, 67.6%, and 72.2% of correct classification were obtained for the SHERR, CLO, and NAP populations, respectively. The posterior membership probabilities <90% suggested admixed genotypes in the populations (Figure 6-3D). The results of the UPGMA dendrogram based on Provesti's genetic distance are consistent with the results obtained with both AMOVA and DAPC; it reveals two main clusters, CA and Quebec, with the latter being further subdivided into two other clusters, CLO-SHERR and NAP (Figure 6-3B).

The genetic structure of *P. destructor* populations was also tested using nonparametric DAPC with no a priori population assignment. The comparison of BIC values for 1 to 10 clusters suggests a rapid decrease in the BIC value from k=1 to k=4, suggesting four genetic clusters containing 24, 13, 42, and 24 isolates (Figure 6-4A). However, the plot of the discriminant function shows three clear clusters with some other clusters embedded within them (Figure 6-4B). The first five eigenvalues accounted for 93.4% of the total variation. The UPGMA dendrogram built using Provesti's distance reveals two main clades of *P. destructor* isolates which did not necessarily correlate with population assignment (Supplementary figure 6-6).

The heterozygosity value was significantly (P<0.05) greater for the NAP population (H_{obs}=0.358) than for the SHERR (H_{obs}=0.315) and CLO (H_{obs}=0.314) populations. The standardized association index $\bar{r}_d$ leads to an unambiguous rejection of the null hypothesis of sexual recombination in the four populations, including California (P<0.001) (Table 6-5). When

tested using, as populations, the four genetic clusters detected by DAPC with no a priori population assignment, the null hypothesis of random mating was also rejected. The calculated $\bar{r}_d$ values ranged from 0.0174 to 0.16 (P<0.01), except for cluster 2 for which $\bar{r}_d$= -0.0273 (not significant). These results provide further evidence to reject the null hypothesis of random mating.

For the Quebec dataset (r = 0.177) as well as the targeted dataset (r = 0.239), the null hypothesis of absence of association between genetic and geographic distances was rejected by the Mantel test (Supplementary figure 6-7). The positive sign of the relationship (i.e., the higher one type of distance, the higher the other) indicated genetic isolation by distance. As expected, the Mantel correlogram showed a decrease of the Mantel statistic with increasing geographic distance. At spatial distances <5.25km, the Mantel statistic was significantly (P<0.05) positive (Figure 6-5). At larger spatial distance classes, isolates had higher genetic distances than expected at random (P≥0.05) (Figure 6-5).

6.5 Discussion

To our knowledge, this is the first population genetic study of the obligate biotrophic plant pathogen *Peronospora destructor*. Considering that the sporadic nature of the disease seems to evolve towards a more endemic style and that the disease is difficult to manage, knowing the sources of initial inoculum is crucial—this study aimed at providing evidence for local overwintering of *P. destructor* populations. Isolates obtained from single onion downy mildew lesions collected from the main Quebec onion production areas were analyzed by GBS, following whole genome amplification. Whereas dominant asexual reproduction leads to limited recombination and clonal population structures, local overwintering introduces genetic differentiation and a gradual accumulation of mutations within populations belonging to different environments. Our results support the hypothesis of a clonal population structure with limited but significant differentiation among production zones.

Clonal populations showed high linkage disequilibrium, meaning that the non-random association of alleles from different loci is higher than expected (Slatkin 2008). As expected, the proportion of alleles in perfect linkage was high and occurred over large regions of the *P. destructor* genome, supporting the hypothesis of clonality (Milgroom 2017). High linkage disequilibrium was also reported for *P. Humuli*, known to have both clonal and homothallic reproduction modes (Gent et al. 2019; Gent et al. 2017). Such high linkage disequilibrium may

introduce redundancy, especially for large genomic data from populations with small effective sample size, and this may limit the resolution in identifying informative loci (Bhattarai et al. 2020; Calus and Vandenplas 2018). In our study, most of the variant loci had G'_{ST} values close to 0 and were randomly distributed throughout the genome even after heavy data pruning, which is characteristic of random sequencing errors and abiotic variation sources (Gent et al. 2019). However, after thorough variant filtering, we still found significant genetic differentiation among *P. destructor* populations in southwestern Quebec.

To date, there is very little information about population genetics for *P. destructor*. Nonetheless, our results are consistent with previous Peronosporale studies reporting limited population genetic diversity. In particular, it has been shown that *P. Humuli* populations were weakly differentiated and that eastern American populations tended to be genetically clustered. In contrast, western American populations were less differentiated (Summers et al. 2015b). Wallace and Quesada-Ocampo (2017) suggested that *P. Humuli* populations were less differentiated than *P. Cubensis* populations, and their results were confirmed by Gent et al. (2019). Low genetic differentiation was reported for Australian populations of *P. viticola*, while populations from North America, identified as the center of origin, were shown to have higher genetic diversity (Taylor et al. 2019).

Low genetic diversity is generally considered to be an indicator of recently introduced or re-emerging plant pathogens in a given region (Grünwald and Goss 2011; Grünwald et al. 2016). In his monograph, Yarwood (1943) reported the important impact of onion downy mildew on Californian onion seed production, mentioning that epidemics were irregular in frequency and variable in severity. *Peronospora destructor* was sporadically reported in North America (i.e., New York State, Province of Ontario) in the 1970s and 1980s and gave rise to the most important outbreaks since the 1950s in 1977-1979 (Hildebrand and Sutton 1982; Smith et al. 1985). Since then, *P. destructor* has been encountered only sporadically in these regions. In 1999, *P. destructor* was reported in sweet onion for the first time in Georgia (Langston and Sumner 2000). This report was followed by two severe epidemics in that region in 2007 and 2012 (Parkunan et al. 2013). In southwestern Quebec, the scenario was different: important epidemics were reported between 1989 and 1993, followed by 11 consecutive years with almost no observed symptoms (Van der Heyden et al. 2020b). Since the mid-2000s, however, there has been a re-emergence of *P. destructor*, with outbreaks reported every year and occurring earlier each year, suggesting that the

pathogen has become endemic, unlike other production areas in North America (Van der Heyden et al. 2020b). Thus, the limited genetic diversity observed in our study support a recent re-emergence of *P. destructor* in southwestern Quebec.

Despite a predominant clonal mode of reproduction, the analysis of GBS data revealed significant genetic differentiation between the geographical regions studied, including southwestern Quebec and California. The results obtained with the DAPC applied using a priori defined populations showed that the California isolates are clustered together and are more genetically distant than the isolates collected from southwestern Quebec. These findings were expected since there is no, or little plant material or seeds exchanged between these two regions. Our results also showed that the Napierville and Sherrington isolates clustered together, while the Ste-Clothilde isolates were less differentiated. These results are supported by the UPGMA dendrogram built from Nei's genetic distances, which first classified populations in two groups (Quebec and California) and then the first group in two subgroups (Ste-Clothilde and Sherrington; Napierville).

Results of the AMOVA indicated a significant effect of the region and field on genetic variation when sampling was regional (Table 2) or targeted (Table 3), which implies that the pathogen persists over time (Gent et al. 2019). Moreover, the pairwise AMOVAs showed genetic differentiation between the same populations, whether with the regional or targeted dataset. Similar results were found for *Ps. humuli*, which exhibited low but significant genetic differentiation at a finer scale, with almost 20% of the genetic variance associated with hop yards all located in western Oregon (Gent et al. 2019). Concerning other factors that might contribute to shape the population structure, we observed no effect of *Allium* species (*A. cepa*, *A. fistulosum* or *A. aggregatum*), unlike other Peronosporales such as *Ps. cubensis* that showed host-related genetic differentiation (Wallace et al. 2020). The AMOVA results suggested a limited but significant effect of year on genetic differentiation, which might indicate occasional influx of new genotypes from adjacent producing regions or the presence of sexual reproduction within the populations.

The re-emergence of onion downy mildew in southwestern Quebec has been associated with warmer weather conditions in autumn and winter, suggesting the overwintering of *P. destructor* inoculum (Van der Heyden et al. 2020b). However, the form in which *P. destructor* survives from one season to the next remains poorly understood. The carryover of *P. destructor* as mycelium in

dormant bulbs has been proposed as one mode of overseasoning (Hildebrand and Sutton 1980; Yarwood 1943). Systemically infected onions used for planting or left in the field after harvest could be the source of seasonal epidemics. Warmer weather conditions could also favor volunteer plants' survival and consequently help maintain the inoculum in the region, which is supported by the limited genetic diversity and clonal population structure that we observed.

A solely asexual mode of reproduction can hardly explain regional onion downy mildew epidemics in southwestern Quebec (Van der Heyden et al. 2020b). In our study, although genetic diversity between muck soil basins was significant, a greater genetic diversity was observed within a specific basin. Such genetic structure in *P. destructor* populations provides evidence supporting the role of local sources of inoculum to initiate onion downy mildew epidemics. High levels of linkage disequilibrium are generally considered an indicator of clonality. However, in addition to a lack of recombination, linkage disequilibrium can also be caused by selection, population admixture, or random genetic drift (Milgroom 2017). In our study, because the standardized index of association $\bar{r}_d$ was significantly different from zero, we rejected the null hypothesis of random mating for the four populations. When tested against the genetic clusters identified with no a priori defined population, the null hypothesis of random mating was also rejected, indicating that linkage disequilibrium was not a function of population structure but arose from a mixed mode of reproduction (Koenick et al. 2018). In addition, the observed differences between DPAC results obtained with and without a previously defined population also point towards population admixture.

The role of random mating and oospore production in *P. destructor* is insufficiently documented, especially in North America. It is generally accepted that the production of oospores is possible, and it is known that they do not occur regularly, can survive for a surprisingly long period (up to 25 years), and need a very long maturation period (up to four years) before they can germinate (McKay 1957; McKay 1937; Yarwood 1943). McKay (1957) stated that oospores were not produced every year, but they could be found in enormous numbers when they were. Thus, rare events of sexual recombination leading to the production of oospores could constitute an important inoculum reservoir that could persist locally for several years. Hence, it is likely that *P. destructor* has a predominantly asexual mode of reproduction, with occasional sexual reproduction events.

It is not known whether *P. destructor* is heterothallic or homothallic, but it is known that the amount of heterozygosity is low in homothallic organisms, especially when populations are established for several generations (Goodwin 1997), and in populations with a strictly asexual mode of reproduction (Goodwin 1997; Van Poucke et al. 2021). However, the results obtained in this study showed that *P. destructor* displayed significant levels of heterozygosity, indicating that it would be heterothallic. These results are consistent with the levels of heterozygosity observed in other heterothallic oomycetes species. For example, the observed levels of heterozygosity were 0.37 on average for *P. effusa* (Lyon et al. 2016) and 0.21 for *P. belbahrii* (Thines et al. 2020), whereas it varied between 0.01 and 0.14 for different heterothallic *Phytophthora* species and between 0.00 and 0.06 for homothallic species (Goodwin 1997).

Airborne *Phytophthora spp. and Peronospora* spp. sporangia are known to travel very long distances, but it is accepted that the majority of the produced inoculum is deposited locally over short distances (Aylor and Taylor 1983; Aylor 1986; Aylor et al. 2001; Corredor-Moreno and Saunders 2019; Fall et al. 2015b; Gent et al. 2009; Granke and Hausbeck 2011). Our Mantel correlogram results support the hypothesis that most of the inoculum is dispersed over short spatial distances. The exponential-like decrease showing higher Mantel statistic values in the first spatial distance classes suggests that there are spatial patches of genetic similarities, perhaps smaller than the grain of our study (*i.e.,* within the muck soil basins) (Diniz-Filho et al. 2013). Limited migration can significantly affect gene flow, leading to a local decrease in genetic diversity. The limited genetic diversity observed in *P. destructor* combined with significant diversity within populations and the fact that these patches of genetic similarity were observed could result from a recent founder event (Goodwin 1997). These results are consistent with the presence of a symptom-free period of nearly ten years observed in southwestern Quebec between 1993 and 2004, followed by a gradual increase in the magnitude of ODM epidemics (Van der Heyden et al. 2020b).

In conclusion, the results obtained in this work can serve as a baseline estimate to describe the genetic diversity and population structure of *P. destructor* and thus contribute to the understanding of Peronosporale ecology. Because of limited numbers of isolates, we could not pinpoint the origin of the initial population but could provide plausible population structures and reproduction modes. Keeping in mind that population genetic analysis provides only indirect evidence of sexual or asexual reproduction modes, we conclude that the genetic structure of *P. destructor* populations in

southwestern Quebec is consistent with the accumulation of mutations leading to slow population divergence in time and space, while occasional sexual reproduction events also occur. Other factors, such as fungicide resistance, may contribute to shape the population structure, and these should be included in further studies (Vogel et al. 2020). Using a finer sampling scale in future research would help confirm the genetic similarity of neighboring spatial patches and searching and finding oospores would be valuable to understand the role of overwintering inoculum. In addition, a comparative genomics study of isolates belonging to the four populations that we studied would be necessary to deepen our knowledge of the organism. Finally, our results should be considered for the management of *P. destructor* in onion production, especially for defining priority areas for intervention when the first symptoms are reported, crop rotation planning, and crop residue management.

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6.7 References for Chapter 6

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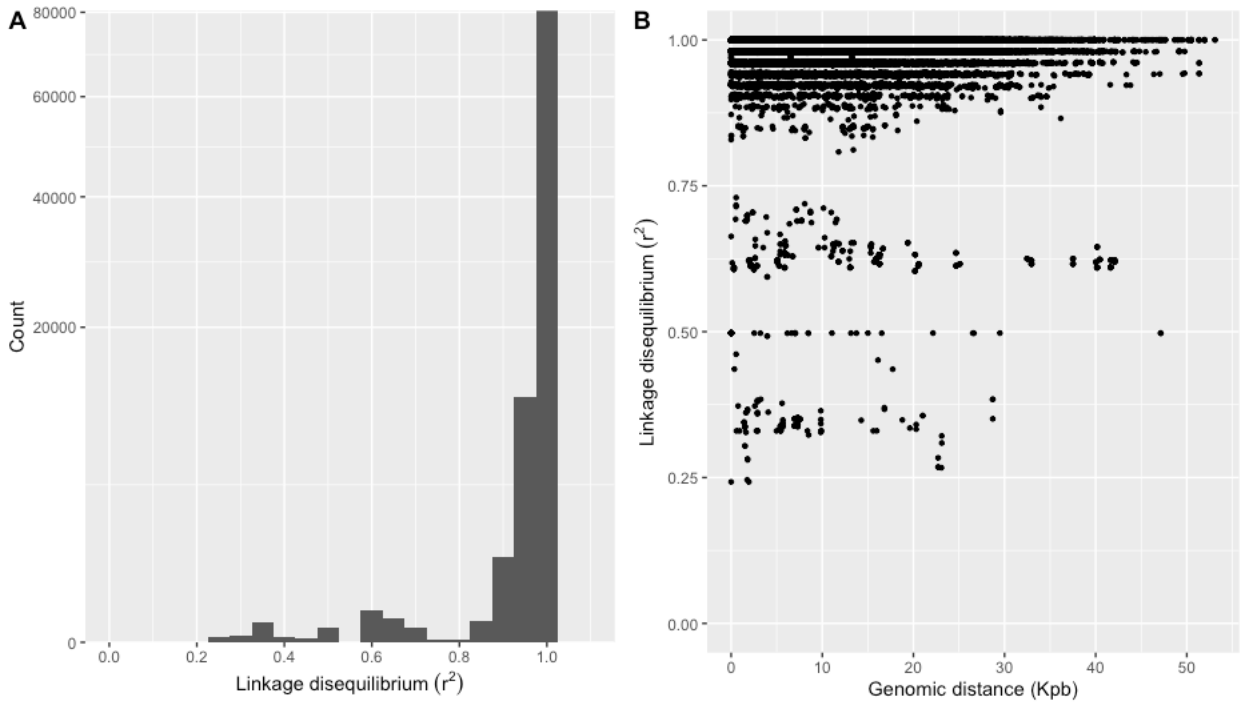


Figure 6-1. Histogram showing the amplitude of linkage disequilibrium (r^2) between the different loci in *Peronospora destructor* isolates (A) and distribution of linkage disequilibrium as a function of genetic distance between associated loci (B).

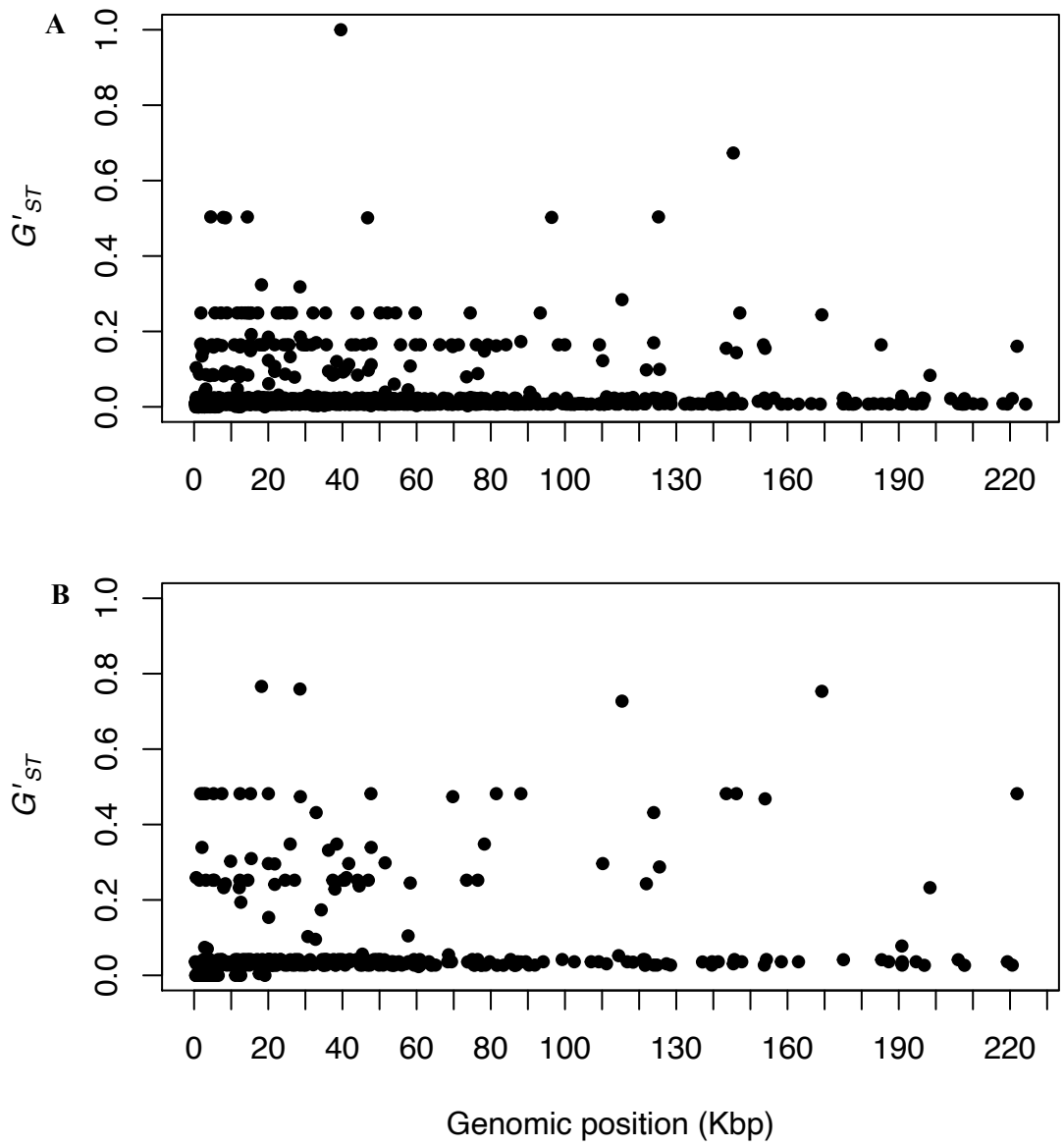


Figure 6-2. Manhattan plots showing the distribution of the G'_{ST} statistics as a function of genomic position constructed with A) the complete data set and B) the targeted subset. These results showed that most of the variants had G'_{ST} values near 0 (no or low differentiation), while a reasonable subset of well-differentiated variants was also present in the data.

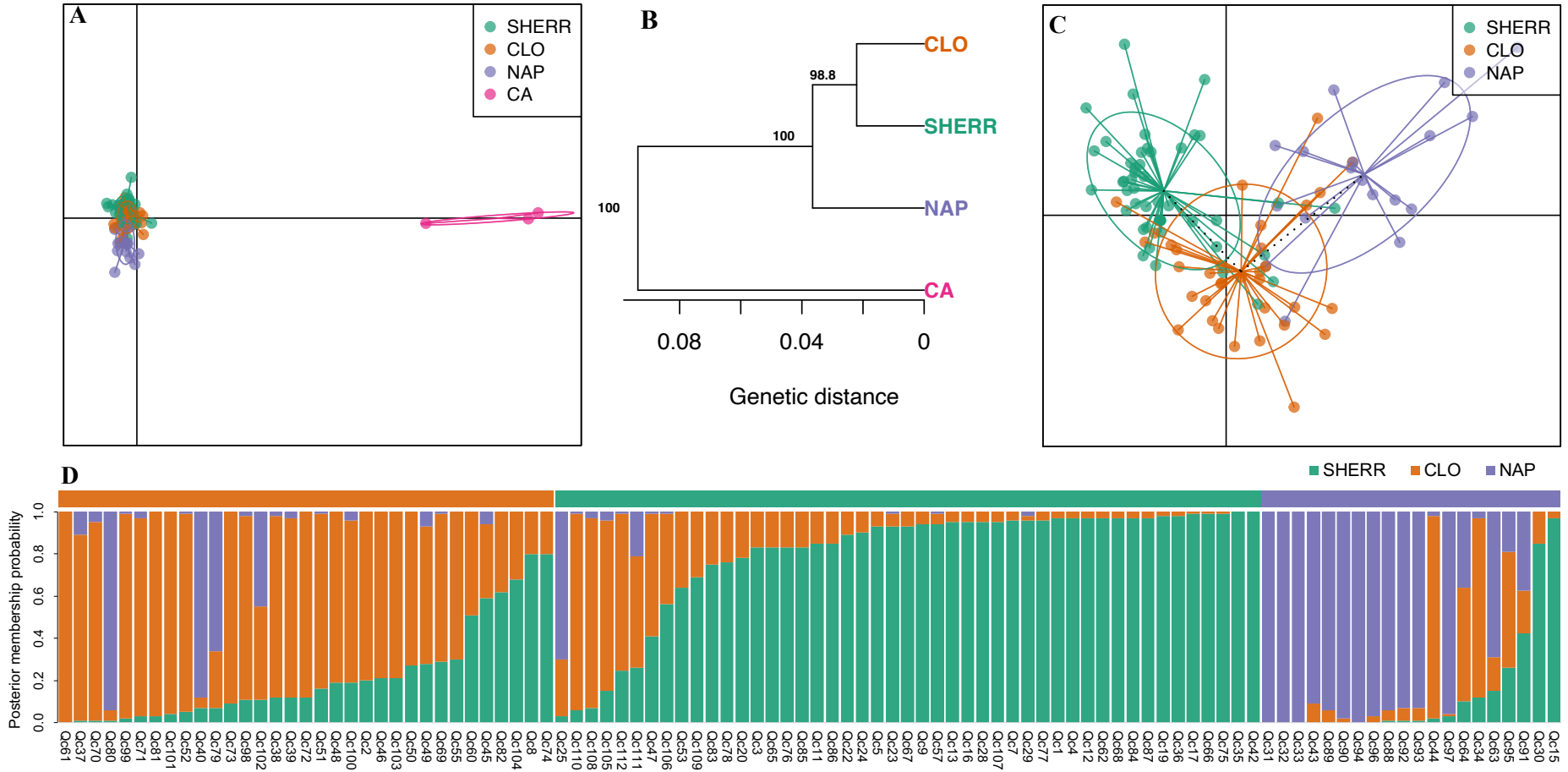


Figure 6-3. Discriminant analysis of principal components (DAPC) conducted with a priori population assignment.In A), the analysis was conducted on the complete datasets with 19 principal components and 2 discriminant functions, chosen following a cross-validation procedure. In B), dendrogram of the relationships among *P. destructor* populations based on Provesti's genetic distance. In C), the analysis was conducted on the Québec dataset, with 25 principal components and 2 discriminant functions, chosen following a cross-validation procedure. In D), a bar graph shows the probability that each isolate belongs to a designated population.

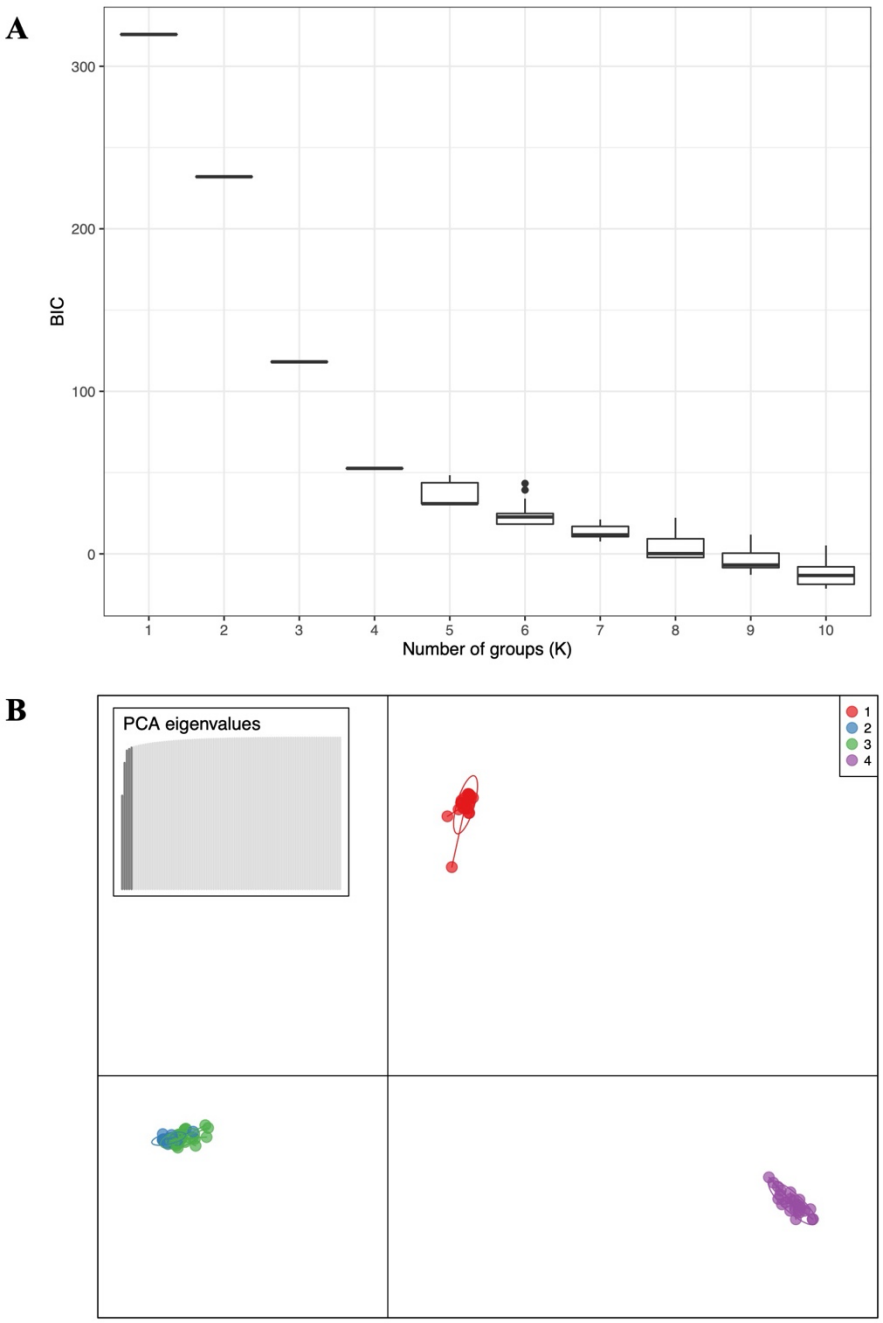


Figure 6-4. Discriminant analysis of principal components conducted with no a priori population assignment.A) Boxplot of Bayesian information criterion obtained for different number of clusters from 999 permutations, indicating the presence of four genetic clusters, B) scatter plot of the discriminant functions representing the main four genetic clusters of *P. destructor* identified by DAPC.

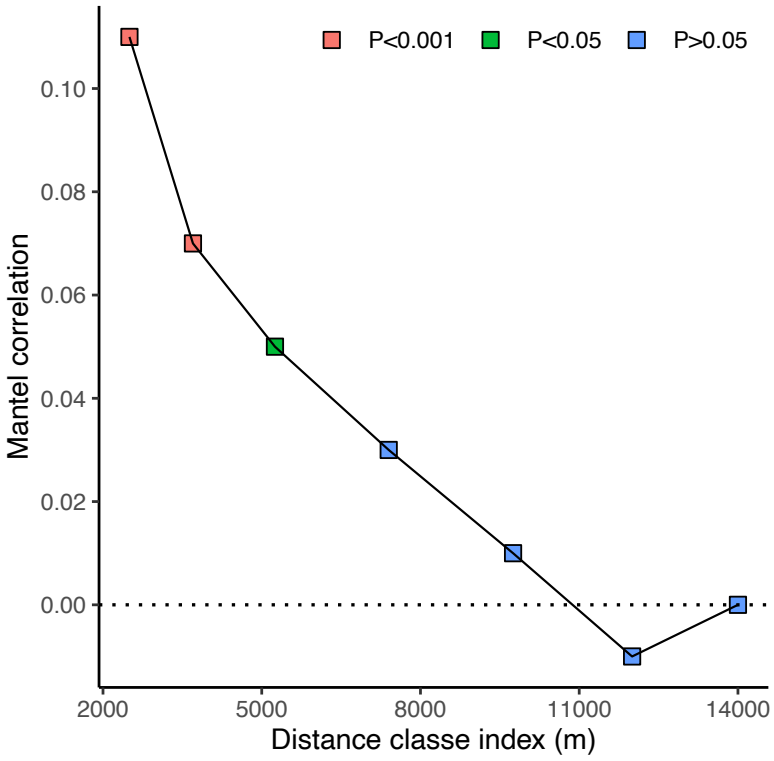


Figure 6-5. Mantel correlogram plotting the correlation coefficient (*r*) between genetic distance and geographic distance in 7 distance classes. The red and green squares indicate a significant correlation while the blue squares indicate that the correlation is not significant.

Table 6-1. Description of the isolates of *Peronospora destructor* kept after quality filtering.

Sample	Year	Grower	Field	CROP	Location[a]	Latitude	Longitude
Qc1	2016	VANW	32	Dry_bulb	SHERR	45.11681	-73.31416
Qc2	2016	BARA	CAMS1	Dry_bulb	CLO	45.15027	-73.40735
Qc3	2016	LECL	30	Bunching	SHERR	45.12344	-73.38038
Qc4	2016	BARR	B5A	Bunching	SHERR	45.09575	-73.35803
Qc5	2017	VANW	20	Dry_bulb	SHERR	45.11662	-73.30671
Qc7	2016	VANW	52	Dry_bulb	SHERR	45.13151	-73.34419
Qc8	2016	BARR	AB1	Bunching	CLO	45.17563	-73.40075
Qc9	2017	VANW	51	Dry_bulb	SHERR	45.13151	-73.34611
Qc11	2016	SOLE	20	Dry_bulb	SHERR	45.11090	-73.38294
Qc12	2017	VANW	31	Dry_bulb	SHERR	45.11420	-73.31577
Qc13	2017	GUEJ	G5	Dry_bulb	SHERR	45.11228	-73.32520
Qc15	2017	VANW	71	Dry_bulb	NAP	45.11505	-73.25197
Qc16	2016	BARR	TMB5	Bunching	CLO	45.15516	-73.40779
Qc17	2018	VANW	52	Dry_bulb	SHERR	45.13114	-73.34353
Qc19	2017	ZUMS	3	Bunching	SHERR	45.13662	-73.37155
Qc20	2017	ZUMS	D2	Bunching	SHERR	45.12199	-73.36379
Qc22	2016	VANW	51	Dry_bulb	SHERR	45.13044	-73.34417
Qc23	2017	GUEJ	B1	Dry_bulb	SHERR	45.12267	-73.37911
Qc24	2017	ZUMS	D7	Bunching	SHERR	45.12047	-73.36288
Qc25	2017	GUEJ	B4	Dry_bulb	SHERR	45.12085	-73.37868
Qc28	2017	GUEJ	G1	Dry_bulb	SHERR	45.11314	-73.32696
Qc29	2017	GUEJ	L5	Dry_bulb	SHERR	45.12881	-73.38487
Qc30	2016	VANW	60	Dry_bulb	NAP	45.11515	-73.25109
Qc31	2016	DELF	C48	Dry_bulb	NAP	45.16394	-73.12679
Qc32	2017	DELF	B36	Dry_bulb	NAP	45.17559	-73.12484
Qc33	2019	DELF	B30	Shallot	NAP	45.17559	-73.12484
Qc34	2019	DELF	B30	Shallot	NAP	45.17583	-73.12418
Qc35	2019	GUEJ	G1	Dry_bulb	SHERR	45.11207	-73.32627
Qc36	2019	GUEJ	G1	Dry_bulb	SHERR	45.11257	-73.32737
Qc37	2019	JSTC	3	Dry_bulb	CLO	45.15072	-73.41980
Qc38	2019	JSTC	3	Dry_bulb	CLO	45.15022	-73.42092
Qc39	2019	LAFI	5	Dry_bulb	CLO	45.14529	-73.44826
Qc40	2019	LAFI	5	Dry_bulb	CLO	45.14492	-73.44770
Qc42	2019	VANW	8	Dry_bulb	SHERR	45.10591	-73.30955
Qc43	2019	VERN	221	Dry_bulb	NAP	45.13999	-73.27191
Qc44	2019	VERN	221	Dry_bulb	NAP	45.14076	-73.26942
Qc49	2019	JSTC	J4	Dry_bulb	CLO	45.13986	-73.43418
Qc50	2019	JSTC	J4	Dry_bulb	CLO	45.13987	-73.43606
Qc52	2019	JSTC	J4	Dry_bulb	CLO	45.13913	-73.43502
Qc53	2019	ZUMS	ND2	Bunching	SHERR	45.12224	-73.36353
Qc45	2016	JSTC	J4	Dry_bulb	CLO	45.13913	-73.43502
Qc46	2019	REMR	R31	Bunching	CLO	45.15657	-73.38100
Qc47	2019	ZUMS	ND2	Bunching	SHERR	45.12224	-73.36353
Qc48	2019	REMR	R31	Bunching	CLO	45.15680	-73.38147
Qc51	2019	JSTC	J4	Dry_bulb	CLO	45.14079	-73.43529
Qc55	2019	BARR	TMB2	Bunching	CLO	45.15443	-73.40884
Qc57	2019	BARR	ORR2	Bunching	SHERR	45.08856	-73.34918
Qc60	2019	JSTC	LABBE1	Dry_bulb	CLO	45.13882	-73.43336
Qc61	2019	JSTC	LABBE1	Dry_bulb	CLO	45.14196	-73.42873
Qc62	2019	VANW	5	Dry_bulb	SHERR	45.11398	-73.30016
Qc63	2019	VANH	J3	Dry_bulb	NAP	45.12609	-73.23789
Qc64	2019	VANH	J3	Dry_bulb	NAP	45.12582	-73.23847

Qc65	2019	VANW	8	Dry_bulb	SHERR	45.10601	-73.30834
Qc66	2019	VANW	8	Dry_bulb	SHERR	45.10547	-73.30847
Qc67	2019	VANW	8	Dry_bulb	SHERR	45.10531	-73.30927
Qc68	2019	VANW	8	Dry_bulb	SHERR	45.10635	-73.30901
Qc69	2019	REMR	M1	Bunching	CLO	45.17042	-73.39599
Qc70	2019	REMR	M1	Bunching	CLO	45.17078	-73.39497
Qc71	2019	REMR	M1	Bunching	CLO	45.16991	-73.39576
Qc72	2019	JSTC	3	Dry_bulb	CLO	45.14960	-73.42096
Qc73	2019	JSTC	3	Dry_bulb	CLO	45.14941	-73.42337
Qc74	2019	JSTC	3	Dry_bulb	CLO	45.14895	-73.42228
Qc75	2019	GUEA	PB4	Dry_bulb	CLO	45.14633	-73.37494
Qc76	2019	BARR	GF28	Bunching	CLO	45.13927	-73.40280
Qc77	2019	LECL	9_11	Bunching	CLO	45.14633	-73.37494
Qc78	2019	LECL	9_11	Bunching	CLO	45.14633	-73.37494
Qc79	2019	LAFI	6_7	Dry_bulb	CLO	45.14591	-73.45108
Qc80	2019	LAFI	6_7	Dry_bulb	CLO	45.14562	-73.45117
Qc81	2019	LAFI	6_7	Dry_bulb	CLO	45.14495	-73.45029
Qc82	2019	LAFI	6_7	Dry_bulb	CLO	45.14501	-73.44979
Qc83	2019	GUEJ	L1	Dry_bulb	SHERR	45.13449	-73.37605
Qc84	2019	GUEJ	B1	Dry_bulb	SHERR	45.12173	-73.37968
Qc85	2019	GUEJ	B4	Dry_bulb	SHERR	45.12044	-73.37927
Qc86	2019	GUEJ	B4	Dry_bulb	SHERR	45.12143	-73.37844
Qc87	2019	GUEJ	B4	Dry_bulb	SHERR	45.12138	-73.37919
Qc88	2019	DELF	B30	Shallot	NAP	45.17623	-73.12471
Qc89	2019	DELF	B30	Shallot	NAP	45.17608	-73.12562
Qc90	2019	DELF	B30	Shallot	NAP	45.17515	-73.12522
Qc91	2019	DELF	B30	Shallot	NAP	45.17437	-73.12486
Qc92	2019	DELF	B30	Shallot	NAP	45.17432	-73.12570
Qc93	2019	DELF	B30	Shallot	NAP	45.17353	-73.12524
Qc94	2019	DELF	B30	Shallot	NAP	45.17352	-73.12627
Qc95	2019	DELF	B30	Shallot	NAP	45.17284	-73.12630
Qc96	2019	DELF	B30	Shallot	NAP	45.17406	-73.12657
Qc97	2019	DELF	B30	Shallot	NAP	45.17437	-73.12486
Qc98	2019	LAFI	5	Dry_bulb	CLO	45.14512	-73.44828
Qc99	2019	LAFI	5	Dry_bulb	CLO	45.14567	-73.44899
Qc100	2019	LAFI	5	Dry_bulb	CLO	45.14612	-73.44887
Qc101	2019	LAFI	5	Dry_bulb	CLO	45.14529	-73.44873
Qc102	2019	LAFI	5	Dry_bulb	CLO	45.14558	-73.44837
Qc103	2019	LAFI	5	Dry_bulb	CLO	45.14580	-73.44934
Qc104	2019	LAFI	5	Dry_bulb	CLO	45.14589	-73.44903
Qc105	2019	GUEJ	G1	Dry_bulb	SHERR	45.11244	-73.32758
Qc106	2019	GUEJ	G1	Dry_bulb	SHERR	45.11188	-73.32755
Qc107	2019	GUEJ	G1	Dry_bulb	SHERR	45.11192	-73.32688
Qc108	2019	GUEJ	G1	Dry_bulb	SHERR	45.11132	-73.32650
Qc109	2019	GUEJ	G1	Dry_bulb	SHERR	45.11154	-73.32603
Qc110	2019	GUEJ	G1	Dry_bulb	SHERR	45.11099	-73.32563
Qc111	2019	GUEJ	G1	Dry_bulb	SHERR	45.11111	-73.32485
Qc112	2019	GUEJ	G1	Dry_bulb	SHERR	45.11161	-73.32531
CA1	2019	CA	2019	CA	CA	-	-
CA2	2019	CA	2019	CA	CA	-	-
CA3	209	CA	2019	CA	CA	-	-

[a] CLO = Sainte-Clotilde; NAP = Napierville; SHERR = Sherrington

Table 6-2. Results of the analysis of molecular variance (AMOVA) conducted with *Peronospora destructor* isolates from the arbitrary sampling carried out in Quebec (regional dataset).

AMOVA	df	Sum of squares	Mean squares	σ^2	Variance component (%)	*P*-value
Between regions	2	150.374	75.187	3.066	17.93	0.002
Within regions	66	926.149	14.032	14.032	82.07	
Total	68	1076.524	15.831	17.099		

Table 6-3. Results of the analysis of molecular variance (AMOVA) conducted with *Peronospora destructor* isolates collected from the three heavily infested field in southwestern Québec (targeted dataset).

AMOVA	df	Sum of squares	Mean squares	σ^2	Variance component (%)	*P*-value
Between fields	2	187.672	93.836	6.988	23.98	0.005
Within fields	28	620.410	22.158	22.158	76.02	
Total	30	808.082	26.936	29.145		

Table 6-4. Pairwise index of differentiation for the regional, targeted and Québec datasets.

Population pair	Regional		2016-2018		2019		Targeted		Quebec[a]	
	Φ_{PT} [a]	*P*-value	Φ_{PT}	*P*-value	Φ_{PT}	*P*-value	Φ_{PT}	*P*-value	Φ_{PT}	*P*-value
NAP-SHERR	0.284	0.006	0.486	0.008	0.408	0.009	0.176	0.039	0.150	0.011
NAP-CLO	0.006	0.292	0.127	0.171	0.084	0.196	0.110	0.120	0.026	0.275
SHERR-CLO	0.153	0.004	0.025	0.440	0.499	0.001	0.345	0.006	0.314	0.0001

[a] Φ_{PT} is the pairwise index of population differentiation.

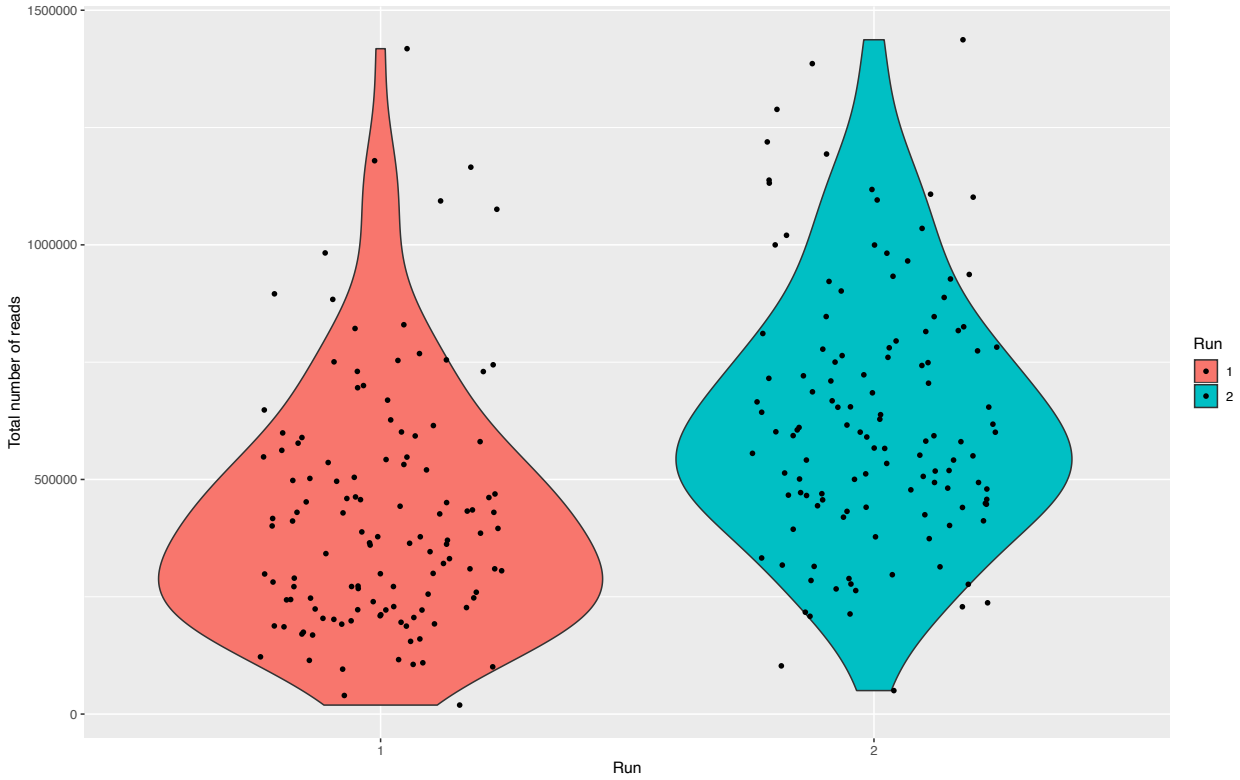
Table 6-5. Summary statistic for genetic diversity and linkage measurements for *Peronospora destructor* populations.

Population	**Heterozygosity** [a]	**Median pairwise genetic distance** [b]	$\bar{r}_d$ **(*P*-value)** [c]
SHE	0.315 (0.280)	0.129	0.314 (0.01)
CLO	0.314 (0.292)	0.146	0.252 (0.01)
NAP	0.358 (0.343)	0.183	0.306 (0.01)
CA	0359 (0.259)	0.129	0.739 (0.01)

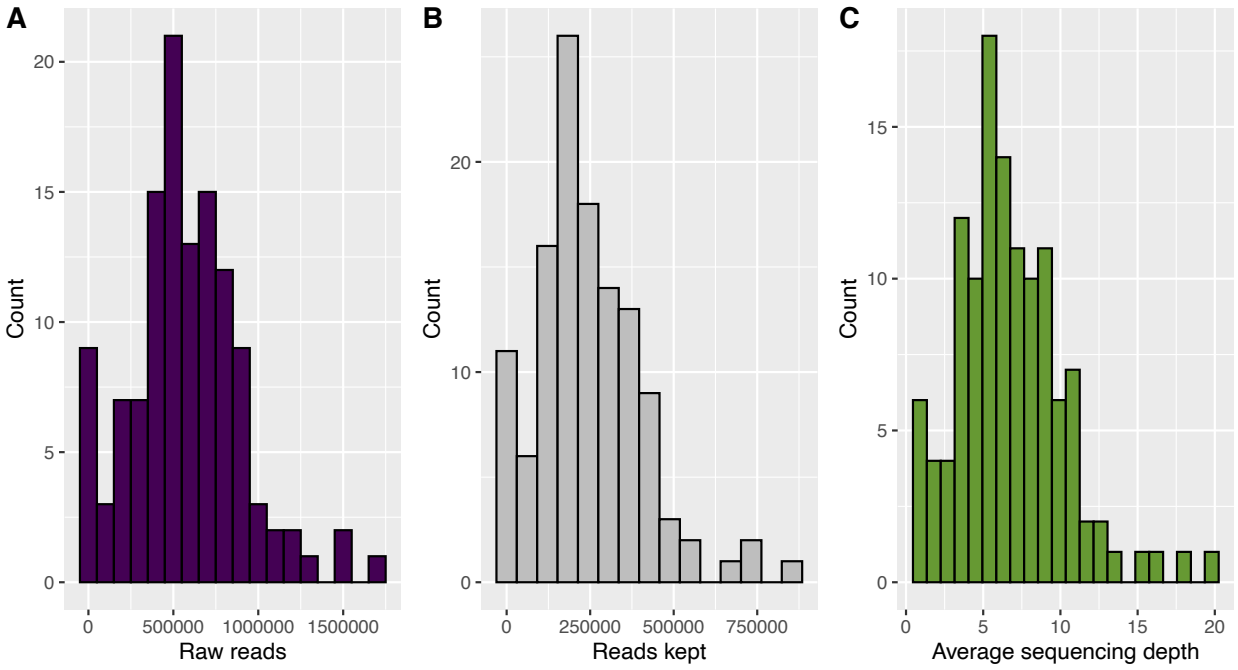
[a] Values in parentheses are expected heterozygosity.

[b] Provesti's pairwise genetic distance.

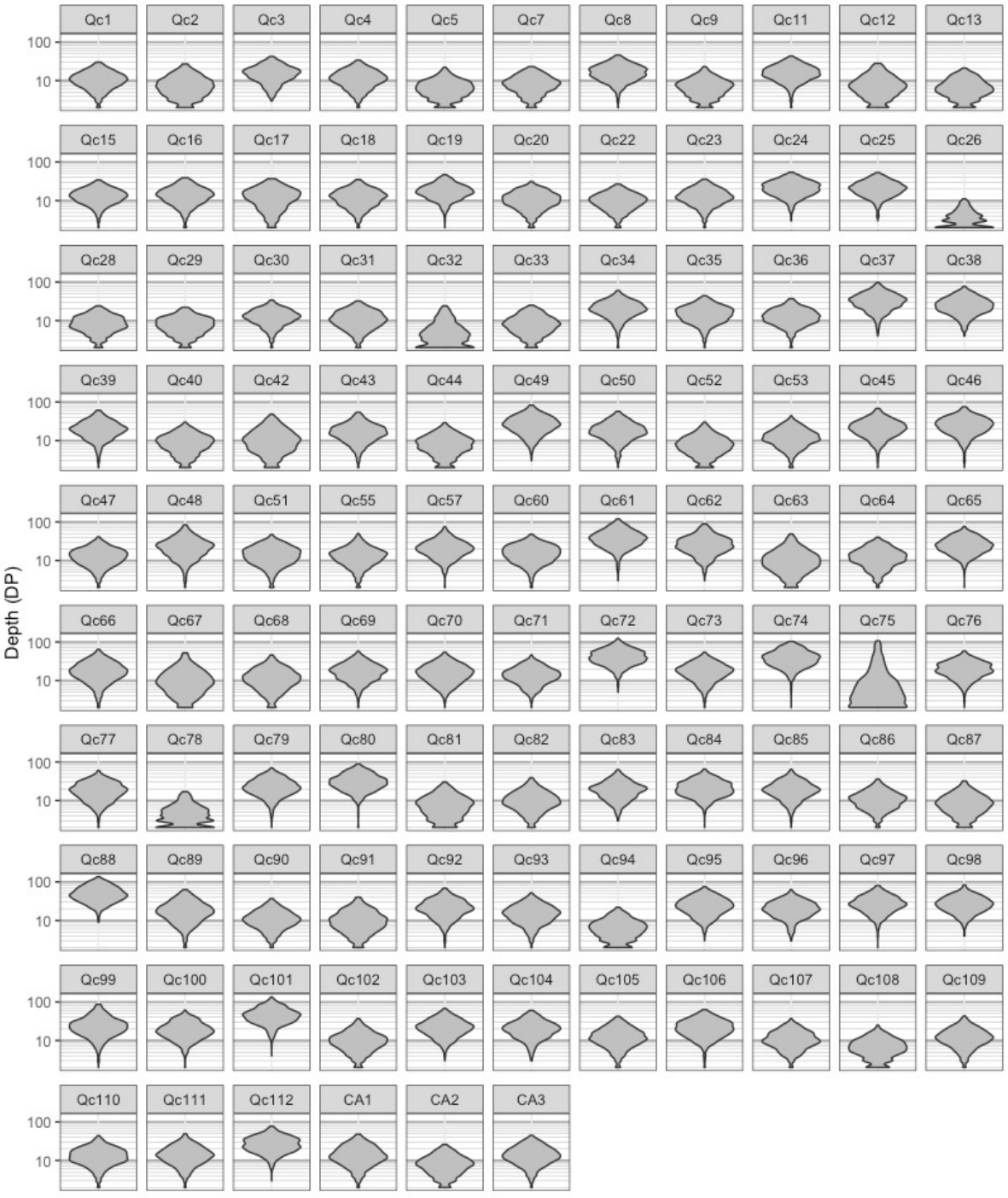
[c] standardized index of association



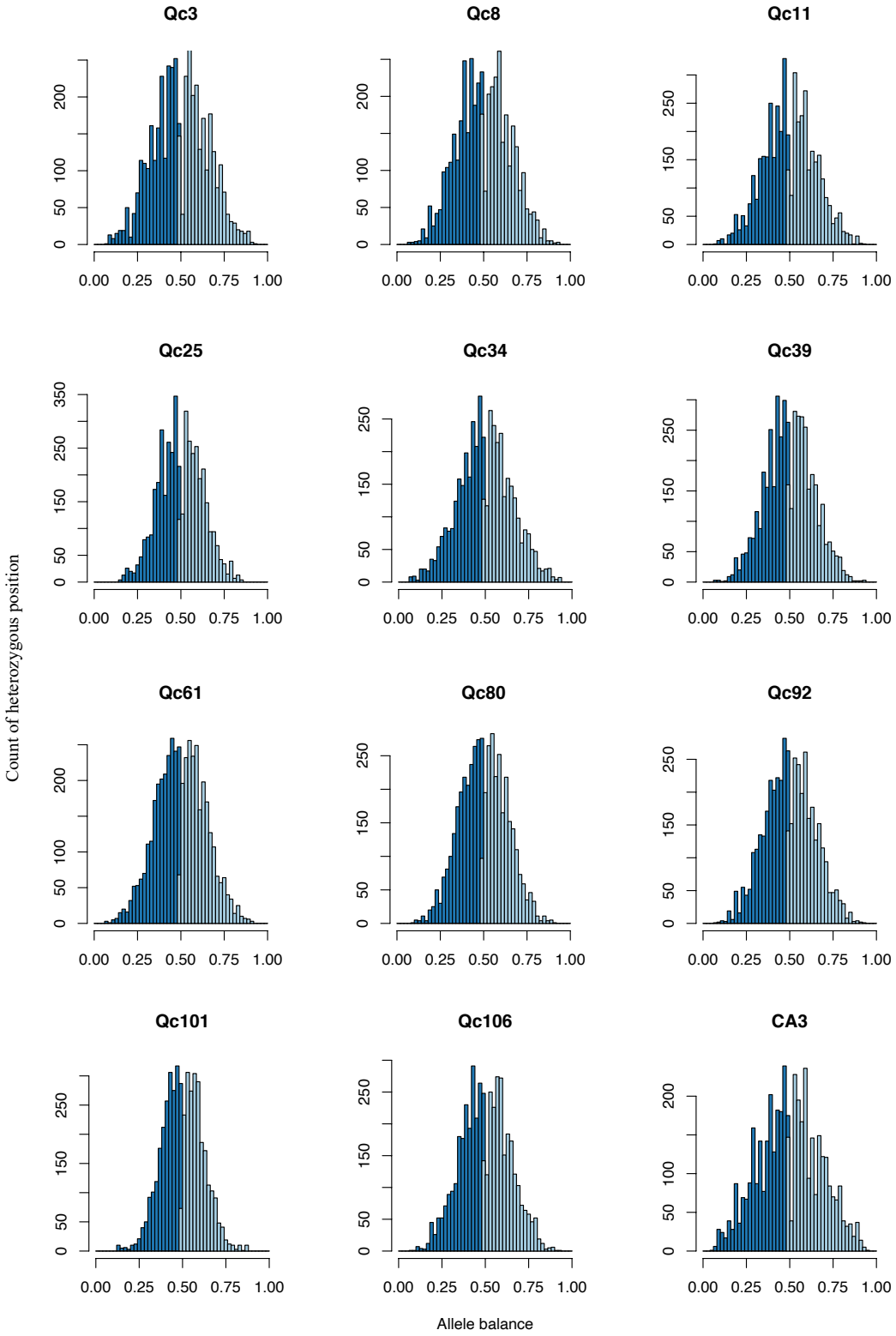
Supplementary figure 6-1. Violin plots showing the distribution of the total number of reads per sample for the two Ion Proton runs (two chips per run) performed in this study.



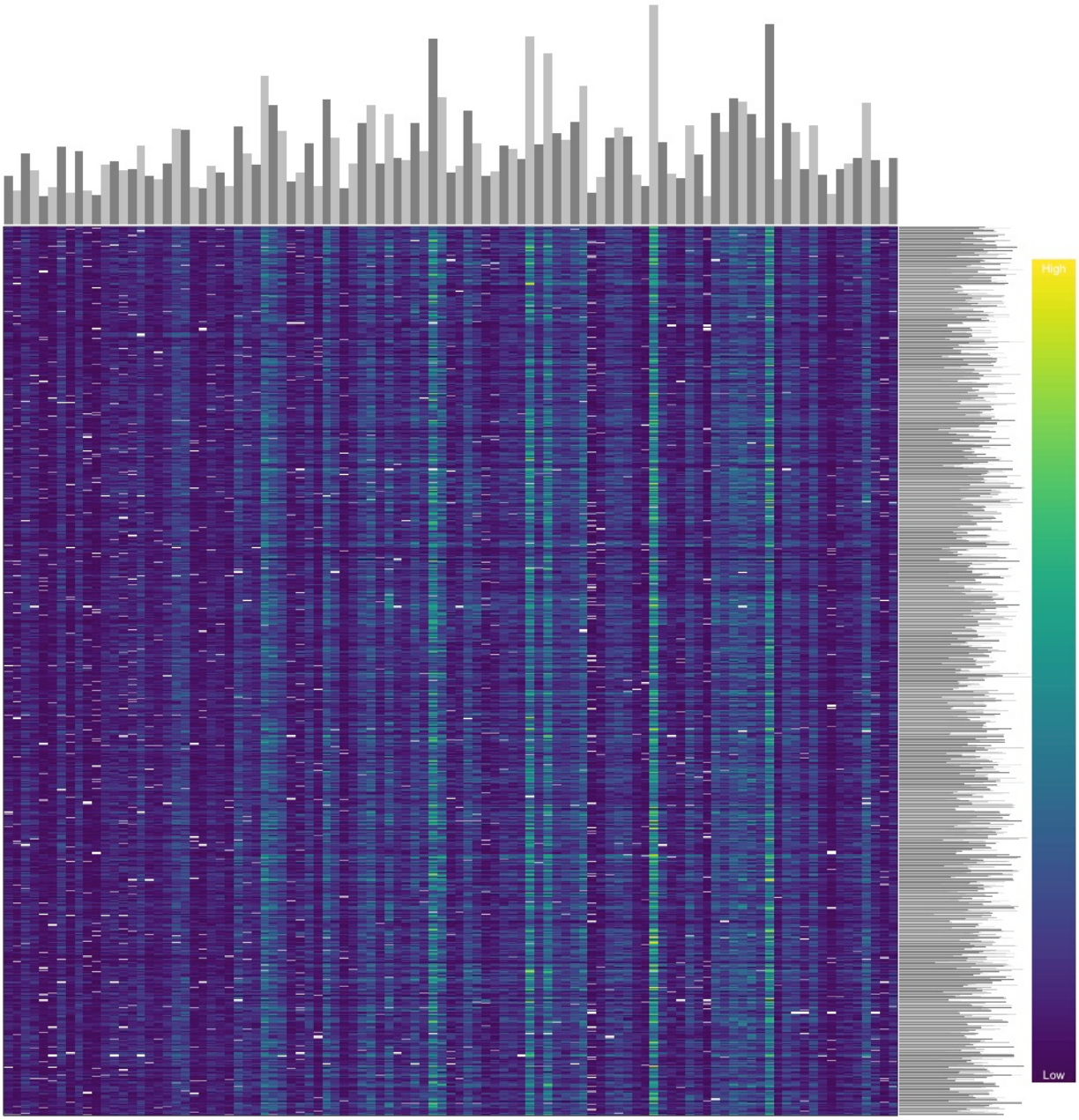
Supplementary figure 6-2. Post sequencing statistics. Distribution of A) the number of raw reads per sample, B) the number of reads kept after alignment to the reference genome and C) the average sequencing depth per sample after alignment to the reference genome.



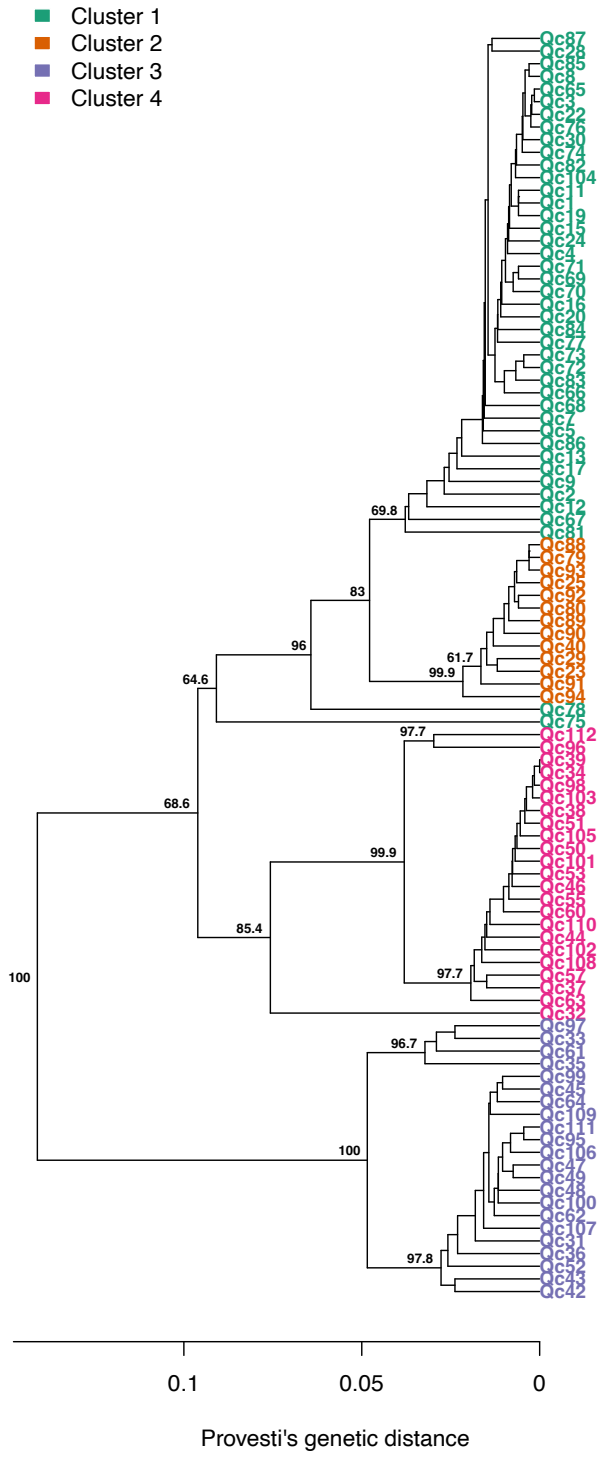
Supplementary figure 6-3. Violin plots showing the distribution of sequencing depth among the loci for each isolate after the filtering steps.



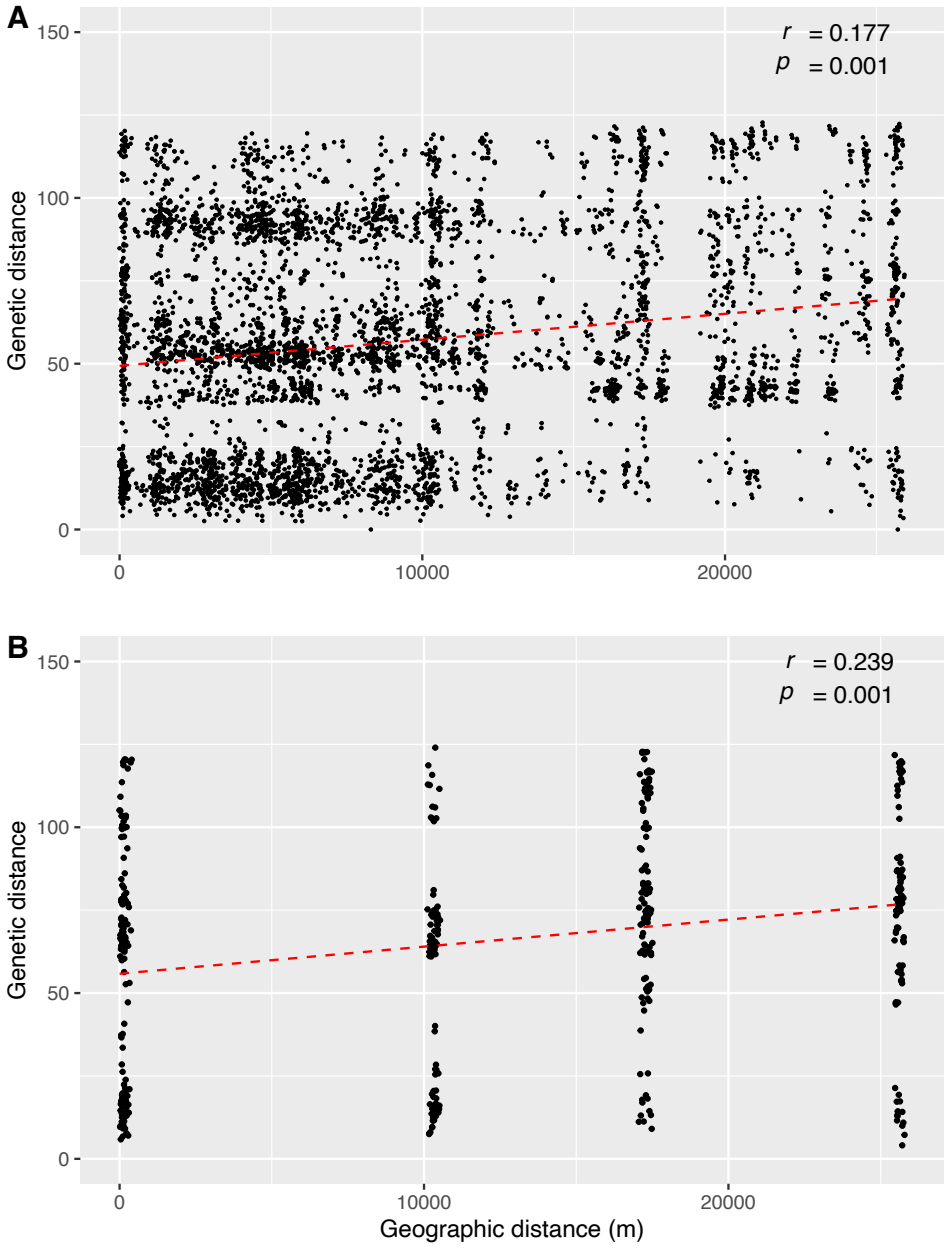
Supplementary figure 6-4. Example of the distribution of allele balance at heterozygous positions for selected *Peronospora destructor* isolates.



Supplementary figure 6-5. Heat map showing the mean sequencing depth for each sample (columns) and for each of the 1,340 retained loci (lines) after the LD filtering step.



Supplementary figure 6-7. UPGMA dendrogram for the Quebec isolates based on Provesti's genetic distance.Isolate colors correspond to the genetic clusters identified by DAPC.



Supplementary figure 6-8. Relationship between genetic and geographic distances (Mantel test)between *Peronospora destructor* isolates for A) the Quebec subset and B) the targeted subset.

Supplementary table 6-6. Results of the analysis of molecular variance (AMOVA) conducted with *Peronospora destructor* isolates from the arbitrary sampling carried out in Quebec (regional dataset).

AMOVA	df	Sum of squares	Mean squares	σ^2	Variation	P-value
Between CROP	2	27.434	13.717	-0.361	-1.94	0.5805
Within CROP	66	1250.023	18.940	18.940	101.94	
Total	68	1277.456	18.786	18.579		

Supplementary table 6-2. Results of the analysis of molecular variance (AMOVA) conducted with *Peronospora destructor* isolates from the arbitrary sampling carried out in Quebec (regional dataset).

AMOVA	df	Sum of squares	Mean squares	σ^2	Variation	P-value
Between YEAR	3	115.904	38.635	1.654	8.47	0.0414
Within YEAR	65	1161.552	17.870	17.870	91.53	
Total	68	1277.456	18.786	19.524		

7 General discussion and conclusions

7.1 Summary

In this thesis, the overarching hypothesis was that the impact of climate change in southwestern Quebec, with warmer temperatures in the fall and milder winters, favoured survival and overwintering of *P. destructor*, modifying the ecology of *P. destructor* and therefore the epidemiology of onion downy mildew. This project focused mainly on the strong interactions between the environment and the pathogen, especially regarding what occurs outside the growing seasons. Using a population biology approach, which combines epidemiology principles and population genetics, the long-term population dynamics of *P. destructor* in southwestern Québec was investigated.

In Chapter 4, ODM epidemics were characterized from a long-term perspective to identify changes in the pathogen epidemiology that are difficult to perceive on shorter time scales. Indeed, if climate change affects ODM epidemics, one can expect that some parameters of these epidemics (e.g., disease onset, intensity, frequency) will change with time. Thus, the first step in this research was to characterize the seasonal outbreaks from 1987 to 2017 to determine whether they were more frequent and occurred earlier. An observational dataset containing 31 years of weekly scouting records was used to achieve this. By dividing the dataset into three decades, it was possible to determine that the regional ODM incidence was significantly higher during 2007-2017 compared with 1987-1996 and 1997-2006. Moreover, for these same periods, the first symptoms occurred on average one month earlier and the 10% disease onset two weeks earlier. These findings were instrumental for the remainder of the study, as they provided the first evidence of changes in ODM epidemiology. Then, using weather data obtained from Environment and natural resource Canada, it was shown that the environmental factors that most influence regional disease incidence and severity were those related to overwintering (i.e., temperature and rainfall at harvest the previous fall, winter coldness, solar radiation, and disease incidence the previous year). Given the significance of these trends, it was concluded that the production and survival of overwintering structures might play an important role in seasonal ODM development, either from soilborne oospores or from inoculum carried in crop residues.

Inoculum carryover from one season to another can vary from region to region and from season to season. The initial inoculum responsible for seasonal epidemics may come from other production areas further south that begin production earlier, from crop residues, or as soilborne oospores. In Chapter 5, a molecular-based assay enabling the detection of *P. destructor* airborne and soilborne inoculum was developed. First, several closely related species were sequenced for the ITS regions, and the sequences obtained were aligned with sequences from public databases. In this way, a discriminating region was identified on ITS2, which allowed the design of a specific primer and probe set. Then, after adding an internal control system, validation was performed with artificially and naturally infested samples. Airborne inoculum levels were found to be consistent with disease incidence, but more importantly, *P. destructor* inoculum was found in soil samples collected in the spring from fields with varying crop rotation lengths. In addition to providing a molecular tool for monitoring inoculum, this research is among the first to present evidence of resting inoculum in soils, reinforcing the hypothesis of local overwintering.

In Chapter 6, following the results obtained in the previous chapters, the genetic structure of *P. destructor* populations was then investigated. To do so, genotyping by sequencing was performed on isolates collected in southwestern Quebec from 2016 to 2019, along with isolates from California as an outgroup. First, it was shown that the structure of the observed populations was characteristic of clonal populations, with an important linkage disequilibrium and a relatively low genetic diversity. The analyses conducted in this study showed that the structure of the populations was not shaped by the host species and that the year effect was limited, but that the genetic differentiation was instead associated with a geographical effect. Moreover, significant patterns of isolation by distance were identified. Finally, the observed levels of heterozygosity indicate heterothallism. Together, these results suggested that the genetic structure of *P. destructor* populations in southwestern Quebec is consistent with the accumulation of mutations leading to slow population divergence in time and space, while occasional sexual reproduction events also occur. Thus, this study was the first to describe the genetic structure of *P. destructor* populations and provided a significant contribution to our understanding of ODM epidemiology.

7.2 The polyetic nature of onion downy mildew caused by *P. destructor.*

Although the importance of survival between growing seasons has been acknowledged in previous studies (Madden and van den Bosch 2002; Tack and Laine 2014; Heesterbeek and Zadoks 1987), it is an aspect that is often neglected in plant pathology. However, inoculum survival between seasons and the proximity of overwintering inoculum sources are as essential for disease emergence as reproduction cycles during the growing season (Willocquet et al. 2020). In their fundamental work on botanical epidemics modelling, Heesterbeek and Zadoks (1987) provided a theoretical framework where epidemics are divided into three sub-processes called zero-, first- and second-order epidemics. Zero-order epidemics are limited to a single field and a single growing season, while first-order epidemics are defined as the spread of a disease over a larger area, including several fields but still limited to a single growing season. Finally, second-order epidemics are defined when the disease spreads over a large area (up to an entire continent) and several consecutive growing seasons (Heesterbeek and Zadoks 1987). Zero-order epidemics usually go unnoticed, while only second-order ones have a polyetic component. Thus, it is the ability of the pathogen to survive between growing seasons and to establish itself over the long term that differentiates first- and second-order epidemics. The results presented in Chapter 3 showed this irregularity in establishing seasonal outbreaks between 1987 and 2007 (Figures 4.1-2). In contrast, the epidemics became more frequent and severe beyond this period, indicating a change that may correspond to the establishment of long-term populations and mark a transition between first and second-order epidemics.

Typically, in southern Quebec, onion cultivation extends from early May (day of the year (doy) ~ 120, sowing) to early September (doy ~ 244, harvest). However, the onion begins to fall from mid-August (doy ~ 227), and from this stage on, the onion dries out, and the amount of green foliage susceptible to downy mildew decreases rapidly. During the 1987-1996 period, onion downy mildew was present in five out of ten years but only appeared late, on average on day 213 (Figure 4-2), just ten days before onion top down. By arriving so late, epidemics remain confined to a few fields, and the pathogen can hardly establish itself (first-order epidemics). After the last outbreak of that decade, onion downy mildew was absent (or undetectable) for six consecutive years. After that, the disease was only reported in 2000, 2003, and 2004, but in less than 1% of the fields and without resulting in an epidemic (zero-order epidemics). It is only since 2005 that the

disease became present every year, and since 2007 that the epidemics started to spread regionally (second-order epidemics). As epidemics move from first- to second-order, the disease onset can be expected to occur earlier in the seasons as the disease becomes increasingly established (Hannukkala et al. 2007). The results presented for *P. destructor* in Chapter 4 are consistent with this expectation as the first observation date, 10% and 50% disease onset decreased significantly over the years (Figure 4-2A). Although it is impossible to identify a precise transition, it is clear that onion downy mildew in southwestern Quebec experienced a succession of zero- and first-order epidemics before becoming polyetic.

When a pathogen is not endemic to a region or territory, its emergence may require a series of migration events before the invasion is successful (Willocquet et al. 2020). However, when conditions are not adequate for maintaining pathogen populations outside the growing season, epidemics are irregular and depend solely on pathogen migration and environmental conditions during the growing season (Willocquet et al. 2020). Conversely, when the pathogen is endemic, epidemics result from what happens during and between growing seasons. Besides, the transition to an endemic disease could involve either a change in the population or a difference in the environment. The results presented in Chapter 4 showed that the most recent onion downy mildew epidemics (2007-2017) were influenced mainly by conditions related to production and survival of overwintering inoculum (temperature and rainfall at harvest the previous fall, winter coldness, solar radiation, and disease incidence the previous year), again suggesting a transition to being polyetic.

Significant differences were identified between periods of the dataset for weather variables, including winter hardiness, fall temperatures, and precipitation during harvest, suggesting that these changes in ODM epidemiology may be related to climate change. The effects of climate change have already been reported for other oomycetes, notably for *P. infestans*. In Finland, for example, Hannukkala et al. (2007) demonstrated, by investigating potato late blight epidemics from 1933 to 1962 and from 1983 to 2002, that the onset of outbreaks tends to occur two to four weeks earlier at the end of the second period and that the effect of soil inoculum was not significant until the late 1990s. These changes in potato late blight epidemic patterns were primarily associated with more conductive climates toward the end of the 1990s. A similar trend was also reported in Sweden from the mid-1990s (Lehsten et al. 2017).

Such changes in the patterns of ODM distributions have implications at different levels. For policymakers, the prospect of such an epidemiological transition should influence the allocation of research resources to help anticipate epidemics and develop mitigation strategies. For growers, knowing that the inoculum could persist beyond the growing season implies a change in farming practices, more precisely, the planning of crop rotations, crop residues, and volunteer plants. In addition, since the disease appears earlier in the season, the initiation and conduct of protective treatment programs must be adapted. Finally, these results emphasize the importance of an effective surveillance program for agronomists and crop specialists. This surveillance can be done through conventional scouting activities, spore trapping, and soilborne inoculum monitoring. The last two above-mentioned methods rely, among other things, on precise and specific monitoring tools allowing the identification and quantification of the inoculum in various types of samples.

Real-time qPCR is one the golden standard methods among the tools tailored for precise inoculum monitoring. However, the only other tool available for *P. destructor* detection was based on monoclonal antibodies and had a detection limit of 500 sporangia, limiting its potential in biomonitoring to detect infected plant tissue (Kennedy and Wakeham 2008; Salcedo et al. 2021). The work presented in Chapter 5 represented the very first molecular tool to detect both airborne and soilborne inoculum. The achieved sensitivity (1 sporangium/m^2 of air and 10 sporangia/g of dry soil) and specificity was comparable to that found in the literature (Carisse et al. 2020; Bello Rodriguez et al. 2020; Dhar et al. 2019; Klosterman et al. 2014; Montes-Borrego et al. 2010; Gent et al. 2009). Furthermore, the ITS regions on which the marker was developed, was considered the universal DNA barcode for fungi and oomycetes. ITS markers showed consistency and reproducibility using qPCR for downy mildews (Crandall et al. 2017; Salcedo et al. 2021) and were successfully used for downy mildews biosurveillance (Montes-Borrego et al. 2010; Gent et al. 2009; Carisse et al. 2020). In addition, an exogenous internal control was successfully added to the system to prevent false negatives and evaluate the presence of qPCR inhibitors that could compromise the amplification efficiency. In addition, unlike other studies, the presence of the internal control did not affect the Cq values for sporangia or ITS copy number standard curves (Kunjeti et al. 2016; Bilodeau et al. 2012).

In Chapter 5, it was shown that *P. destructor* inoculum could be detected from air samples when the qPCR assay is coupled with impaction spore samplers systems, like those implemented for other downy mildews (Bello Rodriguez et al. 2020; Rahman et al. 2020; Klosterman et al.

2014; Gent et al. 2009; Dhar et al. 2019; Kunjeti et al. 2016). The qPCR analyses showed that spore concentrations were low throughout the 2018 season, which was consistent with the average ODM incidence observed. Most of the spore catches were made in early July, but this was followed by a warm, dry period as indicated by the lack of Downcast risk. In 2018, only one sporulation event on July 19 resulted in infection and symptom development 14 days later. In contrast, the 2019 season was likely more conductive, as indicated by the presence of the Downcast risk index throughout the season (Fig 5.7). Sporangia were detected as early as June 4, and concentrations increased in early July as 2018. However, since conditions were and remained favourable until the end of the season, symptoms proliferated accordingly. Thus, although this thesis focused on between-season and overwintering-related phases of the pathogen epidemiology, these results strengthen the premise that epidemics are complex and result from environmental conditions between and during the growing seasons.

The most critical finding presented in Chapter 5 was the presence of *P. destructor* inoculum in soil samples collected in the spring. To validate the test developed in this study, soil samples were taken from fields cultivated with onions the previous year up to four years ago. Although most of the 291 samples were below the quantification limit (below 10 sporangia/g dry soil), 11% were above, and up to 810 sporangia equivalent were found in samples from fields with shorter rotation lengths. Thus, finding *P. destructor* DNA in soils sampled in spring strengthens the hypothesis that it overwinters under the changing climatic conditions of southwestern Quebec. Indeed, since the sampling was done in the spring, it is assumed that most of the short-term survival structures (sporangia and mycelium) where degraded and that only the long-term survival structures, i.e., the oospores, remained. These findings were recently supported by the results of a study conducted in Japan, where *P. destructor* was also amplified from soil using qPCR up to 1600 cells/g of soil (Fujiwara et al. 2021). While the sensitivity of the test evaluated in Fujiwara et al. (2021) was only 100 - 1000 cells/g of soil, the authors conclude that by improving the detection limit, the measurement of soil inoculum could correlate well with disease status.

The mode of over-seasoning and winter survival, the reproductive system, or the migration patterns can largely influence the genetic structure of the populations. Hence, these epidemiological aspects can be inferred from population genetic analyses. The results obtained in Chapter 6 provided evidence of linkage disequilibrium (non-random association of alleles), as

expected for organisms with clonal reproduction systems, indicating that *P. destructor* population of southwestern Quebec contains partially clonal subpopulations (Figure 6-3). This assumption was supported by DAPC and AMOVA, which showed that a significant share of the genetic diversity could be associated with the geographic origin of the isolate. On the one hand, when isolates from California were used, they were found to be highly differentiated from Quebec isolates. On the other hand, significant genetic differentiation was detected between the Quebec subpopulations when the analysis was performed with Quebec isolates only. These findings supported the general hypothesis of this project that *P. destructor* could overwinter in southwestern Quebec and strengthen the results presented in Chapters 4 and 5. Such patterns of linkage disequilibrium and local genetic differentiation have been observed for other *Peronosporaceae* at different scales, including *P. humuli*, which is known to overwinter in the soil and plant crowns at the yard scale (Gent et al. 2019) and *P. tabacina,* which was also reported to be partially clonal (Nowicki et al. 2021). Similarly, for *P. effusa,* the study of SNP data showed that a large part of the genotypic variation found at the field level was associated with asexual reproduction. At the same time, global genetic diversity was influenced by sexual recombination over broader geographic and temporal scales.

We already knew that *P. destructor* was capable of sexual reproduction (McKay 1957; McKay 1937, 1935; Blackwell 1935), but no research had yet determined its mating system. For homothallic organisms, observed heterozygosity levels are expected to be near 0, while fixation index values ($F = 1 - (H_{obs}/H_{exp})$) should be near 1, while under random mating, H_{obs} should be closed to H_{exp} and *F-values* should be near 0 (Goodwin 1997; Halkett et al. 2005). Thus, although a significant linkage disequilibrium was observed for the subpopulations of southwestern Quebec, the H_{obs} values (0.314 to 0.358) are closed to the H_{exp} values (0.280 to 0.343), which suggest that *P. destructor* is an heterothallic organism (Table 6-5). However, the *F* values were slightly negative (-0.13 to -0.04) and significantly different from 0, which suggested a predominantly asexual mode of reproduction. However, care must be taken when interpreting heterozygosity data, as other situations, such as bottle neck, can cause excess heterozygosity. Although the asexual cycle is the predominant form of reproduction of *P. destructor*, sexually produced oospores have been reported (McKay 1957; McKay 1937, 1935; Blackwell 1935). Nevertheless, the sexual phase of the life cycle remains poorly understood and quite controversial as to its role in the disease

cycle. Thus, even if results presented in Chapter 6 bring some light to our understanding of *P. destructor* reproductive systems, more work is needed to fully understand P. destructor mating systems.

The results presented in Chapter 6 demonstrated that genetic diversity is also geographically substructured among *P. destructor* subpopulations. This substructure is characterized by a significant correlation between geographic and genetic distances over relatively short distances (below 5km) (Figure 6-5). Since the rate and distance of dispersal influence genetic divergence, the long-distance spread would lead to lower divergence and, consequently, a weaker correlation between genetic and geographic distances (Kamvar et al. 2015). Thus, these results showed a significant level of isolation by distance and that dispersal could be restricted to short distances. These findings are of some importance, firstly because they indicate that the epidemics probably have a single origin (Kamvar et al. 2015; Nowicki et al. 2021) and secondly, if the sources of inoculum were remote, and only migration was responsible for seasonal epidemics, no such patterns would have been observed. These results are consistent with the dispersal scaling hypothesis (Skelsey et al. 2013), which states that there is a relationship between dispersal and spatial heterogeneity, a theory to which, considering these results, genetic diversity can be included. According to this theory, the extent of dispersal between plots should be maximized when the scale of spatial heterogeneity is neither too fine nor too coarse relative to the characteristics of the species (Skelsey et al. 2013). This has enormous implications for developing management scenarios, such as disrupting landscape connectivity to limit the pathogen's spread or deploying large-scale surveillance networks.

7.3 Strength and limitations

To describe the long-term trends in ODM epidemics, a dataset composed of scouting observations was combined with multivariate statistics (principal components analysis and discriminant analysis). This research is among the few that have used observational data to answer epidemiological questions. For example, such observational data have been used to track the south-north progression of soybean rust in the mid-2000s (Isard et al. 2011; Sikora et al. 2014) or to understand changes in potato downy mildew epidemics in Holland (Zwankhuizen and Zadoks 2002), Sweden and Finland (Hannukkala et al. 2007; Lehsten et al. 2017). Although large-area

observational datasets are rare, some IPM organizations with scouting activities or conducting on-farm experiments are likely to have such observational datasets that can be used to address specific hypotheses. However, these data sources are largely underutilized, although in the era of mega data, this type of data will be increasingly used.

The use of observational data requires to accept to work with data that have been collected for other purposes, which means that it is possible that they cannot be used as-is or contains missing data. Moreover, since such data are not collected following a traditional experimental design, the traditional hypothesis test does not apply. This was the case in Chapter 4, in which the spatial and temporal grain of the data was limited. In fact, the smallest possible level of aggregation was a week for disease progression (progression rate, disease onset, etc.) and an onion growing season for incidence and intensity, covering epidemic development in the MRC des Jardins de Napierville as a whole. However, following the results presented in Chapter 6, we now know that the genetic structure of *P. destructor* populations contains a finer geographic component and that the regional level of aggregation chosen in Chapter 4 may be too coarse. Thus, it would have been interesting to partition the data into the same three subregions used in Chapter 6 (NAP, CLO, and SHE), at least for the epidemics of the last decade. This would likely have allowed us to highlight geographic features associated or not with *P. destructor* genotypes.

The relative scarcity of agronomic data prevented their inclusion in the analysis. It is known, for example, that the number of farms has decreased, and the size of fields has increased in the region. Such changes may have affected disease incidence due to larger fields, narrower crop rotations, and perhaps also due to increasing negligence resulting from unjustified fungicide use. On the weather data side, there were very few federal weather stations (and no provincial stations) that covered the entire period of the dataset: some had data only for the period covering the growing season and did not provide data on winter conditions, while others covered only a few consecutive years. This also limited the statistical approaches that could have been used to analyze the data further. For example, given the number of fields, weeks and years covered by the observational dataset, supervised learning approaches (e.g., random forest, decision trees) could have been used. With the available weather data, however, many observations would have been associated with the same weather condition, which would have weakened the validity of the results.

Following the results obtained in Chapter 4, a molecular marker was developed and validated using many naturally and artificially infested samples to verify if inoculum could be recovered from the environment. The internal nuclear non-coding transcribed spacer (ITS) region was successfully used to develop this sensitive and specific marker. The ITS region is generally considered a universal barcode for fungi and oomycetes and is widely used for marker development for several reasons (Robideau et al. 2011). The ITS region has substantial sequence representation in databases, simplifying the identification by sequence similarity. It is present in multiple tandem repeats per cell, making the regions a desirable target when the required detection level is low. However, ITS regions can exhibit significant heterogeneity between repeats and indels, producing ambiguity on sequence chromatograms or misalignments in addition to variation in copy number per cell and between isolates of the same species (Bilodeau et al. 2012). The alternative barcode rps10 was recently suggested and found to be more appropriate for oomycetes (Foster et al. 2021).

Recent advances in high throughput sequencing (HTS) have changed how we look at diagnosis and detection. Whole-genome sequencing allows the discovery of loci unique to a species or to different genotypes of the same species. However, this is more complicated for obligate biotrophs such as *Peronospora* sp., especially those without a reference genome. However, genome reduction approaches such as GBS allow identifying and developing markers without fully advanced genomes. This kind of approach allows obtaining extremely specific markers and prevents cross-reactivity of the designed assays between related species. Genotyping by sequencing was used in Chapter 6 to investigate the genetic structure of *P. destructor* populations, but not for specific primer design.

The population genetics study presented in Chapter 6 was the first of its kind for *P. destructor*. For the latter, the current availability of a complete genome resource has facilitated this work. This has increased our understanding of the system and generated new reduced genome data. GBS offers a relatively affordable HTS method for genotyping strains. This approach reduces genome complexity by fragmenting DNA with restriction enzymes followed by sequencing of all fragments, which are analyzed to identify variants that can be linked to traits of interest. However, as with other HTS methods, GBS invariably contains sequencing errors that must be accounted for. Thus, although two sequencing runs were used for each isolate, it would have been preferable

to include more technical replicates for some isolates for quality control purposes and ensure confidence in the identified variants. Unfortunately, due to the limited availability of sporangia for this obligate biotroph, only two replicates per strain were used. Therefore, strict retention criteria were used (coverage filtering) to retain only informative SNPs to account for sequencing errors, which resulted in a low number of retained variants.

All population genetics studies begin with a random sampling of the individuals in a population. According to Dr. Grunwald, "the worst thing you can do is take a few samples from your collection and ask colleagues elsewhere in the world to contribute (Grünwald et al. 2017)". In other words, it is essential to structure the sample collection according to what we know about the organism being investigated and the research questions we wish to answer. In any case, perhaps the most critical aspects of an adequate sampling strategy are that the sampled individuals should constitute a random sample of the population they represent and that there should be some hierarchy in the sampling to account for the variation that might occur at different scales. In Chapter 6, isolates were indeed randomly sampled within populations and subsampled from single fields within each population. However, the number of samples collected each season was variable. Although the effect of year was assessable, the analysis of molecular variance could only be done by year for 2019, while 2016-2018 had to be pooled to do so. Thus, the conclusions drawn in Chapter 6 would have been more robust with a larger sample size. Moreover, the sampling was not stratified in time, so it was impossible to investigate the variation between different periods within growing seasons. Another important point in the analysis of population genetics is the use of "outgroup". For this purpose, three *P. destructor* isolates from California were used. However, the addition of isolates belonging to sister taxa would have brought more depth in the analysis and perhaps allowed inferring about the ancestral status of the observed polymorphisms.

7.4 Conclusion

Plant pathogens are highly dependent on environmental conditions to infect their host, especially for fungus and oomycetes-induced diseases (Francl 2007). This relationship between the host, the pathogen, and the environment is quite unique to plant diseases, and it is clear that disease onset results from complex interactions between these three. In this thesis, the concept of the epidemiological triangle was extended to between-season survival to demonstrate how crucial it is to integrate overwintering into our understanding of epidemic dynamics and adaptation of

plant pathogens to environmental heterogeneity. Understanding the ecological and evolutionary factors driving overwintering is necessary to generate predictions about disease dynamics from one season to the next. From an applied standpoint, knowledge of overwintering can be critical for controlling diseases and pests in several agricultural systems and designing sustainable integrated pest management strategies. The influence of overwintering, largely unexplored in plant pathology, is expected to play a similar role in the epidemiology of a wide range of other pathosystems, regardless of whether the pathogen survives as resting structures, in low densities on crop debris or alternate hosts, or saprophytically.

7.5 Future work and perspectives

7.5.1 Comparative analysis of Peronospora destructor genomes.

This project revealed that some genetic variation was present in *P. destructor* populations and that patterns of heterozygosity could suggest a heterothallic mating system. However, there is still little genetic information about *P. destructor*, starting with the molecular basis of the mating system. Recently, the first oomycete Mating-type Locus Sequence was identified in the grapevine downy mildew pathogen, *Plasmopara viticola*, with orthologs locus in *P. infestans*, *P. capsici*, *P. halstedii* and *B. lactucae* (Dussert et al. 2020). This pioneering work could help identify mating systems in *P. destructor*. Hence, conducting a comparative analysis of *P. destructor* genomes could not only shed light on mating systems but also on pathogenicity-related effectors and other secreted pathogenicity-related proteins translocated into the plant cell (e.g., RxLR and LxLFLAK motives) and perhaps on fungicide resistance motives. Furthermore, by including isolates from different regions of the world, it would also be possible to investigate the associated genotypes in greater depth.

7.5.2 Building on metacommunity theory to investigate the influence of biotic interactions in the epidemiology of P. destructor.

As mentioned at various points in this thesis, the environment plays a predominant role in the development of plant diseases both seasonally and between seasons. For the purpose of this thesis, only weather and geographical conditions (abiotic) were used in the "environment vertex". However, it is known that the assembly of microorganism communities (comprising pests) can include a wide range of facilitative, neutral, or antagonistic interactions, which can exacerbate or

inhibit disease transmission, pathogen virulence and survival, and potentially influence disease emergence (Seabloom et al. 2015). Recent developments in the metacommunity theory have shown the importance of biotic interactions in understanding the structure of a system. Accordingly, the structure of metacommunities can be characterized from an external perspective (i.e., coexistence patterns) or an internal perspective (i.e., environmental features of species, spatial autocorrelation, and co-occurrence among species) (Leibold et al. 2021). This can be achieved by using joint species distribution models, a family of multivariate models designed to account for species responding jointly to the environment and each other (Leibold et al. 2021; Ovaskainen and Abrego 2020). Furthermore, third-generation sequencing approaches promise in situ or near in situ sequencing of different environmental samples at low cost, allowing to unravel the diversity of leaves and soil microbiota (Maestri et al. 2019). Thus, with this metacommunity approach combined with third-generation sequencing tools, complex modelling of biotic and abiotic factors could help to characterize further the conditions favouring epidemics, both during and between growing seasons.

7.5.3 Integration of landscape heterogeneity in modelling onion downy mildew epidemics.

Chapter 6 of this thesis found patterns of isolation by distance, suggesting that dispersal patterns may be limited to short distances and that environmental heterogeneity can affect local epidemics. These results also emphasized the need to shift epidemic management and modeling toward a landscape scale. A contemporary approach in theoretical epidemiology is to use SEIR-type compartmental models with individuals classified as susceptible (S), exposed (E), infectious (I), and removed (R). In such compartmental models, the transition between states (S-E-I-R) is formulated as a set of linked differential equations with parameters specific to a given pathosystem, such as the basic reproductive number (R_0), transmission rates, and the probability that spores land on leaves. Traditionally, states are used for a plant or a part of a plant as the basic unit. Yet, this approach has recently been applied to larger basic units (i.e., a field), with a dispersal kernel to estimate the net dispersal probability of pathogens among fields in the landscape, thus incorporating all possible sources and recipient host plants in each pair of fields (Papaïx et al. 2014). Moreover, this kind of spatially explicit compartmental model could incorporate genetic aspects and metacommunities and thus contribute to improving our knowledge and optimizing control strategies.

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