Insights into population structure and epidemiology of *Phytophthora infestans* from Nicaragua

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Cover: Potato and tomato leaves affected by the late blight pathogen *Phytophthora infestans* (Mont.) de Bary.
(photo: Jorge Ulises Blandón-Díaz)

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Abstract

Late blight caused by *Phytophthora infestans* (Mont) de Bary is a constraint to both potato and tomato crops in the northern highlands of Nicaragua. This thesis describes studies on population structure and epidemiology of *P. infestans* from Nicaragua.

The genotypic and phenotypic variation in isolates of *P. infestans* collected in potato and tomato growing areas of northern Nicaragua were analyzed using genotypic (SSR fingerprinting and mtDNA haplotyping) and phenotypic markers (mating type, virulence and fungicide sensitivity). Genotypic markers revealed no polymorphism among the *P. infestans* isolates tested. Phenotypic variation was observed. Nicaraguan population of *P. infestans* is dominated by a clonal lineage of the A2 mating type, Ia mtDNA haplotype and no evidence of genetic population differentiation among potato and tomato isolates was found.

The aggressiveness of *P. infestans* isolates sampled from potato and tomato fields was determined through cross-inoculations experiments. Potato and tomato isolates both had a shorter LP, higher SP, and were more aggressive on tomato leaflets compared to potato ones.

The adequacy of the late blight simulation model LATEBLIGHT (version LB2004) was evaluated under Nicaraguan conditions. During 2007-2008 field experiments were conducted in Nicaragua. The simulation model was considered adequate as it accurately predicted high disease severity in susceptible cultivars without fungicide sprays, and demonstrated a decrease in the disease progress curves with additional fungicide applications, similar to that observed in the field plots. The quantitative relationship between host resistance and the need for fungicide was also investigated using simulations performed with LATEBLIGHT, as well as field trials.

Keywords: pathogen diversity, quantitative pathogenicity, epidemiology, modelling, host resistance, *Phytophthora infestans*, fungicide resistance

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Dedicada

A Jürgen, Jorge Ulises Jr., Lorena y a mi querida familia en Estelí y Managua.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Blandón-Díaz, J.U., Widmark, A.-K., Hannukkala, A., Andersson, B., Högberg, N., and Yuen, J.E. (2011). Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. *Phytopathology*. (Accepted with revisions).

II Blandón-Díaz, J.U., Högberg, N., Grönberg, L., Widmark, A.-K., and Yuen, J.E. (2011). Aggressiveness and genotyping of *Phytophthora infestans* isolates from Nicaragua. (Manuscript).

III Blandón-Díaz, J.U., Forbes, G.A., Andrade-Piedra, J.L., and Yuen, J.E. (2011). Assessing the adequacy of the simulation model LATEBLIGHT under Nicaraguan conditions. *Plant Disease*. (In press).

IV Blandón-Díaz, J.U., Forbes, G.A., Taipe, A., Knutsson, J., Andrade-Piedra, J.L., and Yuen, J.E. Epidemiological significance of the quantitative relationship between host resistance and fungicide usage. (Manuscript).

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Abbreviations

AI	Aggressiveness index
ANOVA	Analysis of variance
AUDPC	Area under disease progress curve
CTAB	Cetyltrimetylammonium bromide
DNA	Deoxyribonucleic acid
EAT	Envelope of acceptance test
GLM	Generalized linear model
H_hr	Hours per day of relative humidity >85%
IP	Incubation period
LA	Lesion area
LB2004	Lateblight model version 2004
LGR	Lesion growth rate
LP	Latency period
LSMEANS	Least-squares means
mtDNA	Mitochondrial DNA
PCR	Polymerase chain reaction
RAUDPC	Relative area under disease progress curve
SA	Sporulating area
SCRI	Scotish Crop Research Institute
SP	Spore production
SR	Sporulation rate
SSR	Simple sequence repeats

1 Introduction

In Nicaragua potato production is concentrated in three northern departments (provinces), namely, Estelí, Jinotega and Matagalpa. The production is dependent on imported seed and is expensive. The potato is grown on hillside lands positioned at altitudes ranging from 700 to 1500 meters above sea level (masl), although it has also been grown in the Sebaco valley located at 400 masl. Under optimal growing conditions, the yields of potato range from 20 to 25 t ha^{-1}. Potato consumption in Nicaragua is about 2727.3 tons per month, except December when it increases to 4090.9 tons. The national production does not meet domestic demand even though the country has potential planting areas (355,233 ha), which could be used for potato production (PFID-F&V, 2005). In 2008 and 2009, Nicaragua produced 33000 metric tons in 2300 ha (FAOSTAT, 2008). The average annual import volume represents 55% of apparent consumption, while domestic production accounts for 45% (PFID-F&V, 2005). As in many countries around the world, the potato crop in Nicaragua is attacked by many pests and diseases that require producers to use large amounts of insecticides, fungicides and bactericides. One of the most important diseases affecting potato crop in Nicaragua is late blight, caused by the oomycete *Phytophthora infestans*. This pathogen also affects tomato, which is grown year round in altitudes ranging from 400 to 1500 masl. The area allocated for the tomato crop is approximately 2000 ha and the yields range from 12 to 18 t ha^{-1} (Berlin and Eitren, 2005). Potato and tomato are grown in adjacent areas increasing the risk of passing the late blight from one crop to another if one of them becomes infected. However, these crops rarely are grown in the same field.

This thesis describes aspects until recently unknown about *P. infestans* in Nicaragua. This oomycete causes late blight disease on potato and tomato

crops. The genus name (*Phytophthora*) in Greek means literally and accurately the "plant destroyer".

Sampling of potato and tomato fields affected by late blight in northern Nicaragua was carried out from 2007 to 2010. From these samples, axenic cultures of *P. infestans* were obtained and used for genotypic and phenotypic characterization of the plant pathogen. Genotypic characterization was done using simple sequence repeats (SSRs, also known as microsatellites) and mitochondrial DNA (mtDNA) haplotyping. The pathogen was phenotypically characterized through mating type determination, virulence and fungicide sensitivity testing. Aditionally, cross-inoculation experiments were performed to determine the level of variation of aggressiveness in *P. infestans* isolates sampled from potato and tomato fields. A slight genotypic variation among the tested *P. infestans* isolates was found. Nonetheless, phenotypic variation (high levels of metalaxyl resistance and race diversity) among tested isolates was shown. Moreover, there seems to be some kind of specialization toward tomato based on the aggressiveness tests (Paper **I** and **II**).

Field experiments during 2007-2008 in two northern regions (Estelí and Matagalpa) of Nicaragua were set up to assess the adequacy of the late blight simulation model LATEBLIGHT (version LB2004) under Nicaraguan conditions. Two susceptible (Cal White and Granola) and one resistant (Jacqueline Lee) potato cultivars were evaluated, without use of fungicide, and with three application intervals (4, 7 and 14 days) of the fungicide chlorothalonil. The simulation model was considered adequate as it accurately predicted high disease severity in susceptible cultivars without fungicide protection, and demonstrated a decrease in the disease progress curves with additional fungicide applications, similar to that observed in the field plots. The model also generally predicted inadequate fungicide control, even with a 4-day spray interval, which also occurred in the field. Lack of adequate fungicide protection would indicate the need for cultivars with higher levels of durable resistance, and that farmers should consider more effective fungicide applications (higher dosages or different chemistries) if susceptible cultivars are used. The LATEBLIGHT model was also used to investigate the quantitative relationship between host resistance and fungicide usage (Paper **III** and **IV**).

2 Background

2.1 An overview of population biology/structure of *Phytophthora infestans* (Mont.) de Bary

In agricultural systems, plants are constantly exposed to a wide range of pathogenic microorganisms, including bacteria, fungi and oomycetes. Fungi and oomycete plant pathogens cause many of the world's most notorious plant diseases, which threaten global food production and consequently food security. Fungi and oomycetes are in general divided into biotrophs, necrotrophs and hemibiotrophs, based on the different strategies they use to colonize plants. Biotrophic plant pathogens establish a nutritional relationship with living host cells; necrotrophic pathogens rapidly kill host plant tissues; and hemibiotrophic plant pathogens have both a biotrophic and necrotrophic phase during its life cycle (Bouwmeester et al., 2009; Dodds and Rathjen, 2010; Schneider and Collmer, 2010).

More than 150 years after the Irish potato famine, *Phytophthora infestans*, the causal agent of late blight disease, continues to be an economically important pathogen of potato and tomato worldwide (Ristaino, 2002). The disease was reported first in 1843 in some northeastern areas of United States, from where it spread to others parts of the country and Canada. In Europe, the disease was reported in Belgium, Holland, Germany, Switzerland, France and Italy in the middle of 1845, spreading rapidly to England, Scotland and Ireland in the same year. In the latter country the pathogen found the optimal environmental conditions for its development and caused devastating epidemics leading to the infamous great potato famine by mid-October 1845, which resulted in ecological and social disaster in Ireland (Peterson, 1992; Ristaino, 2002; Scholthof, 2007). After

the Irish potato famine, the pioneering work of early mycologists in the identification of *P. infestans* and further elucidation that it was the cause and not the effect of the disease known as late blight laid the foundations for the disciplines of Microbiology and Plant Pathology (Judelson and Blanco, 2005; Ristaino, 2002).

The late blight pathogen, *P. infestans*, is a diploid, heterothallic and hemibiotrophic oomycete that poses a real and potential threat not only to economically important crops such as potatoes and tomatoes, but also for tree and shrub species of the family Solanaceae (Bouwmeester et al., 2009; Fry, 2008). The "devastating plant destroyer", *P. infestans*, causes economic losses yearly calculated in multibillion dollars (Haverkort et al., 2008). *P. infestans* can reproduce asexually and sexually. Sexual reproduction in this heterothallic oomycete only occurs when two mating types termed the A1 and A2 outcross. As a result of this, oospores are produced, which can survive in the absence of a host (Drenth et al., 1995; Ristaino, 2002). In locations with only the asexual cycle, *P. infestans* survives as mycelium in infected potato tubers and debris (Ristaino, 2002) and probably also in alternate wild hosts. As a hemibiotrophic plant pathogen, *P. infestans* has both a biotrophic and necrotrophic phase during its life cycle (Bouwmeester et al., 2009). In compatible interactions with potato, the biotrophic phase of *P. infestans* can last from three to five days, after which macroscopic symptoms are evident (necrotrophic phase). In tomato leaves, an extended period of biotrophy has been observed due to a compatible interaction with tomato-specialized isolates (Legard et al., 1995; Smart et al., 2003; Vega-Sánchez et al., 2000).

Prior to the 1980s, worldwide populations of *P. infestans* were dominated by a single clonal lineage known as the US-1 "old" genotype, with the A1 mating type (Fry and Goodwin, 1997b; Goodwin et al., 1994a). In contrast, in the Toluca Valley in central Mexico the A1 and A2 mating types were present in approximately equal frequencies and the populations of *P. infestans* were entirely different from populations in other locations (Fry, 2008; Goodwin et al., 1992). Since the mid-1980s changes in the population structure of *P. infestans* outside Mexico have been reported (Fry and Goodwin, 1997a). These changes brought about the displacement of the 'old' genotypes by 'new' ones, which are characterized by increased fitness and aggressiveness in addition to metalaxyl resistance (Day and Shattock, 1997). The pathogen has been found to be reproducing sexually under field conditions outside its putative center of origin. There are also reports of oospore formation and oospores acting as initial inoculum (Andersson et al., 1998; Lehtinen and Hannukkala, 2004). High population

diversity has been found worldwide in studies conducted using molecular markers (Forbes et al., 1998). The appearance of fitter and more aggressive strains has prompted the development of methods for rapid detection and identification of these strains in order to design and implement control measures (Trout et al., 1997). The development of high-throughput codominant markers can undoubtedly contribute to the understanding of *P. infestans* population biology, epidemiology, ecology, genetics and evolution as a prerequisite for devising appropriate management practices (Cooke and Lees, 2004).

Populations of *P. infestans* have been characterized using a series of genotypic and phenotypic markers. Phenotypically, populations of *P. infestans* have been distinguished through determination of the mating type, virulence spectrum and metalaxyl resistance (Fry et al., 1993). The genotypic characterization of *P. infestans* strains has included the use of allozyme patterns, mitochondrial DNA (mtDNA) haplotype determination, AFLP and RFLP fingerprints with the probe RG57. In a recent study, results from mtDNA haplotyping and RFLP analysis led to the suggestion that multiple migrations of *P. infestans* into China have occurred (Guo et al., 2010). In comparison with the previously mentioned markers, simple sequence repeat markers (SSRs, also referred to as microsatellites) seem to offer the greatest potential across a wide range of applications (Cooke and Lees, 2004). Over the past ten years, SSRs have been developed for the study of *P. infestans* (Knapova et al., 2001; Knapova and Gisi, 2002; Lees et al., 2006). Microsatellite markers have been used recently to infer that multiple introduction events of *P. infestans* have taken place in France and that *P. infestans* populations from this country are composed by two differentiated genetic cluster of isolates (Montarry et al., 2010).

In Latin America, *P. infestans* populations have been extensively studied. In the Toluca Valley in central Mexico *P. infestans* reproduces sexually and the two mating types (A1 and A2) are found in approximately equal frequencies. In other countries of the subcontinent, *P. infestans* appears to reproduce primarily asexually, although both mating types have been found in the same host. For instance, initial studies in Ecuador reported the presence of two clonal lineages (EC-1 and US-1) of the A1 mating type (Forbes et al., 1997). However, further studies revealed the occurrence of the two mating types (A1 and A2) of *P. infestans* sensu lato in the same host (*Solanum muricatum*) (Adler et al., 2002), but the A2 isolate in that study is probably *Phytophthora andina* (Oliva et al., 2010); in Peru an A1 clonal population is reported (Garry et al., 2005; Pérez et al., 2001); in Brazil, the A1 and A2 mating types are found in tomato and potato respectively, but

they have never been found in the same field (Reis et al., 2003). In Uruguay, only the A2 mating type has been found (Deahl et al., 2003); in Colombia, the A1 and A2 mating types have been found in the same host, *Physalis peruviana* (cape gooseberry), although no evidence of sexual recombination has been reported so far (Vargas et al., 2009); in Costa Rica, a clonal lineage of A1 mating type has been reported attacking potato, however, isolates of the A2 mating type has been also found in wild *Solanum* species (Gómez-Alpizar, 2004). In Argentina, *P. infestans* populations seem to be more diverse when compared with other Latin American countries since both A1 and A2 mating types have been found. However, Argentinean isolates of the A2 mating type have been found in a higher frequency and they showed greater aggressiveness and an increased resistance to metalaxyl (Andreu et al., 2010). Clonality of *P. infestans* populations has been also reported from Venezuela, where only the A1 mating type has been reported (Briceño et al., 2009).

A persistent problem in plant-pathogen interactions is poor understanding of the basis of host specificity, that is, what factors determine the taxonomic range of hosts that can be infected by a specific plant pathogen. This is a fundamental question that relates both to the co-evolution of host susceptibility and pathogen virulence. In general, there is very limited knowledge about the genetics and mechanisms involved in host specificity, although host specificity varies among plant pathogens and could be determined by the phylogenetic distance between plants. The host range may include a large number of plant species at one extreme or only a single genotype of a single plant species at the other (Barret et al., 2009; Gilbert and Webb, 2007). The effects inflicted by plant pathogens on their host plants may be qualitative and quantitative and, hence, there are qualitative and quantitative specificity. Qualitative specificity does not allow a particular plant pathogen to infect many hosts, whereas, when the specificity is quantitative, the plant pathogens have a lower performance on certain host plants (Barret et al., 2009).

The host range of *P. infestans*, generally, has been considered to be restricted to two important crops, potato and tomato, various wild species in the genus *Solanum* and also some nonsolanaceous species (Adler et al., 2002; Adler et al., 2004; Erwin and Ribeiro, 1996; Kroon, 2010). Nonetheless, the factors determining this host range remain unknown (Kamoun and Smart, 2005). Some degree of pathogenic specialization of *P. infestans* to potato or tomato has been reported (Fry, 2008; Legard et al., 1995; Oyarzun et al., 1998; Suassuna et al., 2004; Vega-Sánchez et al., 2000; Wangsomboondee et al., 2002). In USA, for example, the US-8

genotype has been detected to occur on potatoes and the US-7 and US-17 genotypes have been recovered from tomatoes (Goodwin et al., 1998). A study in Kenya and Uganda clearly showed that late blight epidemics in potato and tomato were caused by two separate, host-adapted populations of *P. infestans* (Vega-Sánchez et al., 2000). In contrast to this, a recent study carried out in Taiwan showed no host specificity on potato or tomato among *P. infestans* isolates from tomato (Chen et al., 2008). Negative relationships (genetic trade-offs) between qualitative and quantitative traits required to infect one or another host can drive the appearance of pathogenic specialization mediated by antagonistic pleiotropy, in which one or more genes favour pahtogen's performance in one host, but impair its performance in another (Barret et al., 2009; Kawecki, 1998; Pariaud et al., 2009).

Migration events have contributed in shaping the population structure of *P. infestans* in a number of locations around the world and Nicaragua does not appear to be the exception. Deductively, it may be possible to formulate three hypotheses to explain the presence of *P. infestans* on potato and tomato fields in Nicaragua. Two of these hypotheses are related to human-mediated migration events; while the third hypothesis is that *P. infestans* has always been present in Nicaragua. Firstly, it is believed that cultivated potato (*Solanum tuberosum)* was introduced to Nicaragua in the early 1900's, and with it the late blight pathogen, and since then they have coevolved. Troops from United States supposedly brought potato tubers for consumption, some of which fell into the hands of local people who began growing potatoes. Therefore it could be hypothesized that the first populations of *P. infestans* present in Nicaragua belonged to the single "old" clonal genotype (US-1 genotype) of the A1 mating type and the Ib mtDNA haplotype (Fry and Goodwin, 1997a). Moreover, this introduction could have occurred before the second worldwide migration of *P. infestans* that has been suggested to have taken place in the 1980´s. This migration spread new genotypes that were, resistant to phenylamide fungicides and showed an increased aggressiveness to potato (Goodwin et al., 1994b). Secondly, infected potatoes or tomatoes with *P. infestans* could have been carried from northwestern Mexico to Nicaragua in the 1950s. This hypothesis is based on the fact that the mitochondrial haplotypes Ia and IIb have been found in herbarium specimens dating from 1954 and 1956 respectively (May and Ristaino, 2004). The possible route of migration events that led *P. infestans* to Nicaragua is shown in Figure 1. Thirdly, another possibility to explain the presence of *P. infestans* in Nicaraguan potato and tomato fields is that native plants of the *Solanaceae* family could have hosted a pre-existing

population of *P. infestans* long before the introduction of the potato as a crop. Twenty-two genera and 118 species of the *Solanaceae* family are found in Nicaragua. The genus *Solanum* alone contains 45% of the total number of species in this family in Nicaragua (Stevens et al., 2001). The pepper (*Capsicum annuum*) might be the first domesticated plant that served as host to *P. infestans*, since it was grown by the natives when the Spanish conquerors arrived in Nicaragua in the early sixteenth century (www.wikipedia.org).

Figure 1. Possible route of *P. infestans* migration to Nicaragua from United States in the early 1900s (US-1 genotype) and from northwestern Mexico in the middle of the 1950s (US-6 genotype).

2.2 Management of late blight disease

Suggested management strategies for late blight include use of clean seed, elimination of real and potential sources of inoculum (infected cull piles, volunteer potato plants, and wild alternate hosts), fungicides, decision support systems (DSS), intercropping, cultivar mixtures and extended crop rotations (3-4 years) to avoid early infections developed from oospores. Along with these strategies, the use of resistant cultivars against late blight is of utmost importance, especially in locations where environmental conditions are conducive for disease development and potato growers cannot afford the numerous fungicides required to control the disease (Andersson et al., 2009; Fry, 2008; Kirk et al., 2005; Lehtinen et al., 2009; Pilet et al., 2006). Potato growers have relied primarily on fungicide use for late blight control, both in developed and developing countries (Andrivon *et al.*, 2006). However, environmental and health concerns accompanying pesticide use (Shtienberg *et al.*, 1989) combined with the fungicide application costs, has prompted the search for more economically and environmentally sound control measures. Currently, strategies for late blight management aim to reduce the population size and growth rate of the pathogen in order to delay the epidemic onset and subsequently to reduce disease severity (Li *et al.*, 2009).

Three types of resistance against potato late blight have been identified: race-specific resistance (RS), race-nonspecific resistance (RNS) associated with late maturity and race-nonspecific resistance (RNS) which acts irrespective of maturity (Vanderplank, 1957). Race-specific resistance act delaying the epidemic onset without altering the apparent infection rates, whereas race-nonspecific resistance decreases the speed of epidemic progress without influencing the date of initial disease outbreak (Parlevliet, 1979; Vanderplank, 1968). Moreover, race-specific resistance is thought to provide complete protection, but only to certain races of the pathogen species and is governed by a single gene or a small number of related genes. This type of resistance is considered less durable. The race-nonspecific resistance involves multiple genes, provides only partial, but more lasting protection (McDonald and Linde, 2002). For many years, potato breeders have attempted to defeat *P. infestans* and its arsenal of pathogenicity factors (effectors) introducing resistance (R) alleles from *Solanum demissum* (reviewed by Gebhardt and Valkonen, 2001) and more recently from *Solanum bulbocastanum* (Helgeson *et al.*, 1998; Song *et al.*, 2003) two wild potato species indigenous to Mexico. Moreover, some attempts have been done to exploit host potato resistance in order to make a more rationale use of fungicide in a sense to determine the optimal number and timing of

fungicide applications using different approaches (Fry, 1977; Fry, 1978; Fry and Shtienberg, 1990; Kankwatsa *et al.*, 2002; Kirk *et al.*, 2001; Kirk *et al.*, 2005; Shtienberg *et al.*, 1989). In the integration of host resistance and rationale use of fungicides for late blight control, it is important to consider other factors such as weather and socio-economic conditions for a specific location (Crissman *et al.*, 1998; Ortiz *et al.*, 2004). From the epidemiological point of view it is very important to carry out a precise and accurate assessment of host potato resistance and to know the cultivar performance under different agroecological growing conditions to develop and implement environmentally and economically effective strategies aimed at controling late blight. First steps in that direction have been taken (Andrivon *et al.*, 2006; Hansen *et al.*, 2005; Nærstad *et al.*, 2007; Yuen and Forbes, 2009).

For the potato growers of the developing countries, the majority of whom have limited economic resources, the most promising alternative at the moment for late blight management is the use of varieties with elevated levels of resistance and judicious use of low-cost fungicides, such as mancozeb (Grünwald et al., 2002). Nevertheless, the determination of the timing, number and frequency of applications of fungicides in a specific locality, and with a specific cultivar, is a difficult task since it requires many field experiments due to the great variation in weather and available levels of host resistance among regions (Ortiz et al., 2004). Given these circumstances, computerized models for disease simulation can be effective tools in the evaluation of strategies for disease control as they allow preliminary assessment of a number of scenarios involving many variables, including weather conditions, level of host resistance and aggressiveness/virulence of the local pathogen population (Andrade-Piedra et al., 2005a). Disease simulation can thus reduce research costs, since only the most promising strategies would be evaluated in the field (Shtienberg et al., 1989; Shtienberg and Fry, 1990).

Initially, the LATEBLIGHT simulation was used to investigate the effect of rate-reducing resistance on the performance of the protectant fungicide chlorothalonil sprayed at fixed intervals (Bruhn and Fry, 1981). Later, an improved version of LATEBLIGHT (version LB1990) was used to examine several strategies involving the fungicides metalaxyl and chlorothalonil for late blight control and delay of metalaxyl resistance development in pathogen populations (Doster et al., 1990). Details of the model components and descriptions of how simulations are conducted have been described (Andrade-Piedra et al., 2005b; Bruhn et al., 1980; Bruhn and Fry, 1981; Doster et al., 1990; Fry et al., 1991). The most recent version of the

simulation model LATEBLIGHT, LB2004, was developed and validated by Andrade-Piedra et al. (2005b). The epidemiological parameters were measured in three Peruvian varieties infected with isolates of the EC-1 clonal lineage of *P. infestans*, which is dominant in Peru and the Northern Andes (Fry et al., 2009). In that study, the authors only simulated disease in nontreated plots (Andrade-Piedra et al., 2005b). The fungicide sub-model published by Bruhn and Fry (1982a,b) did not give satisfactory results with the data from Peru (unpublished data). The LB2004 version was subsequently used successfully with data from locations worldwide, but again without fungicide applications (Andrade-Piedra et al., 2005c).

Given the need for more effective management strategies against late blight, it was proposed that LATEBLIGHT simulation model version LB2004 could be a useful tool for initial evaluation of disease management scenarios in Nicaragua. However, it was not known if the model would work under Nicaraguan conditions either because of the epidemiological parameters used by Andrade-Piedra et al. (2005a) would not be appropriate for the Nicaraguan cultivars and pathogen population, or for other undetermined reasons. It was also proposed to evaluate the appropriateness of a modified version of the fungicide sub-model. One problem associated with the management of late blight with host resistance in developing countries is the lack of a system for quantifying host resistance. Recently, it was proposed a simple scale for quantifying resistance (susceptibility) that is putatively robust across locations (Yuen and Forbes, 2009).

3 Aims of this study

- To assess the genotypic and phenotypic variation in isolates of *P. infestans* collected in potato and tomato growing areas of northern Nicaragua. In addition, this study also aimed to compare the isolates of *P. infestans* from potato and tomato to determine whether there was differentiation between these two groups of isolates at the genotypic and phenotypic level (Paper **I**).
- To establish whether there were differences in aggressiveness among potato and tomato isolates of *P. infestans*; to determine whether the *P. infestans* population from Nicaragua is formed exclusively by a single clonal lineage (Paper **II**).
- To evaluate the appropriateness of the LATEBLIGHT simulation model for use in Nicaragua. The specific objectives of this field study were: i) to assess the adequacy of the LATEBLIGHT simulation model (version LB2004) for disease management scenario testing under Nicaraguan conditions; ii) to quantify the degree of susceptibility to *P. infestans* in three potato cultivars grown in Nicaragua; and iii) to compare three application intervals of the contact fungicide, chlorothalonil for disease management with these cultivars. (Paper **III**).
- To determine the theoretical relationship between the intensity of fungicide use and level of host plant resistance. An additional objective was to determine an optimum fungicide treatment strategy (timing and number of fungicide applications needed) to control late blight in accordance with the levels of host plant resistance of the cultivars used in this study and the prevailing geographical weather conditions of the experimental sites (Paper **IV**).

4 Materials and Methods

4.1 Sampling and isolation of *Phytophthora infestans* (Paper I and II)

Leaflets of potato and tomato with a single late blight lesion were collected from commercial production and experimental fields in three departments (Estelí, Jinotega and Matagalpa) in northern Nicaragua from July 2007 to January 2010 (Figure 2). In each department five to seven locations were sampled, taking a different number of samples from each location (Table 1). Thirteen tomato and forty-three potato fields (56 fields in total) located in eighteen sites in three northern departments of Nicaragua were sampled. Details about the sampling sites and the number of isolates collected per department and particular location are shown in Table 1. The infected leaflets were washed with distilled water and dried with filter paper. Thereafter, they were individually placed abaxial side up in a sealed Petri dish containing a layer of 1.5% water agar and incubated at 18°C to promote sporulation. When sporulation was observed, the mycelia with sporangia were transferred to a pea agar medium (Flier et al., 2003), amended with antibiotics (0.2 g ampicillin and 10 mg pimaricin L^{-1}) and incubated at 18°C in darkness for a week. Plugs of agar with growing hyphal tips were cut from the colony margins and transferred to Petri dishes with pea agar medium without antibiotics and incubated at 18°C for growth and sporulation. Axenic isolates were maintained on pea agar medium without antibiotics and transferred monthly to fresh medium. Of the sampled material, fifty-four blighted potato and tomato leaflets were preserved as dried material for DNA extraction.

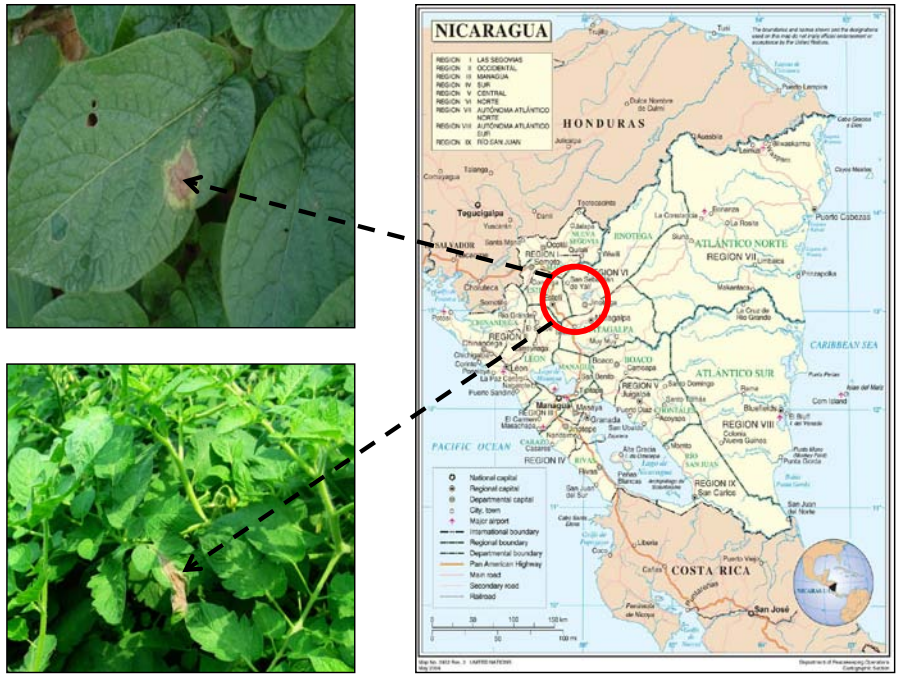

Figure 2. Samples of single lesion potato and tomato leaflets infected with *Phytophthora infestans* were collected from three northern departments of Nicaragua from 2007 to 2010. A total of 248 isolates of *P. infestans* were obtained.

4.2 DNA extraction

Two approaches were used to extract DNA for mitochondrial haplotyping and microsatellite (SSR) analysis depending on whether the sample was stored as lyophilized mycelium or as dried leaflets. Individual pieces of lyophilized mycelium were placed in a 2-mL polypropylene vial containing six glass beads and homogenized in a FastPrep preparation shaker (Precellys 24, Bertin Technologies). DNA was extracted following the protocol provided with the Wizard® Genomic DNA purification kit: protocol for plant tissue (Promega) for isolating genomic DNA from plant tissue. Dried leaflets of potato and tomato infected with *P. infestans* were homogenized for DNA extraction as described for lyophilized mycelium. DNA from dried leaflets was extracted using a cetyltrimethylammonium bromide (CTAB) procedure (Gardes and Bruns, 1993), with exception that 3% CTAB was used.

4.3 Genotypic characterization

Mitochondrial DNA (mtDNA) haplotyping was carried out using a method described earlier (Griffith and Shaw, 1998), with slight modifications. The annealing temperature was increased to 63°C and the primer pairs P2 and P4 were used at a concentration of 0.4 μM. Microsatellite analysis was carried out as described in **Papers I** and **II**. For genotypic characterization, 204 isolates of *P. infestans* from Nicaragua were used.

4.4 Phenotypic characterization

Mating type determination was done using tester isolates of known mating type (A1 or A2) and the unknown Nicaraguan isolates. Mycelial plugs (0.5 cm diameter) of each were placed in petri dishes containing rye pea agar (Lehtinen et al., 2008) at 20°C in the dark. Cultures were examined for oospore formation in the zone of interaction. In the mating type assays 248 Nicaraguan isolates of *P. infestans* were used.

The fungicide sensitivity of the isolates to metalaxyl-M and propamocarb hydrochloride (propamocarb-HCl) and the virulence testing was done using the floating leaf disc method (Lehtinen et al., 2008; Sozzi et al., 1992). For virulence testing, a procedure described earlier (Lehtinen et al., 2008) was followed. Only potato plants with resistance genes were used for virulence tests, since no tomato differentials were available. Mean number of virulence factors per isolate (*Ci*) and race (*Cp*) was calculated (Andrivon, 1994). The *Ci* and *Cp* were separately calculated for the potato and tomato isolates. Moreover, to detect differences among potato and tomato isolates, a *t-test* procedure for the *Ci* and *Cp* values was performed.

For aggressiveness determination, sixteen isolates from potato and fifteen isolates from tomato were used in cross-inoculation assays, .i.e., potato leaflets were individually inoculated with potato and tomato isolates and the same was done with tomato leaflets. The inoculum was prepared directly from artificially infected potato and tomato leaflets. Each isolate-host combination was repeated five times (one leaflet of potato or tomato per Petri dish). Potato or tomato leaflets were placed abaxial face up on the lids of inverted Petri dishes lined with 1.5% water agar in the base and inoculated with a 20 μL droplet of sporangial suspension adjusted to 2×10^4 sporangia mL^{-1} of the appropriate test isolate (potato or tomato). Thereafter, the inoculated leaflets were incubated at 16°C and 16 h day length. The incubation period (IP), latency period (LP), lesion area (LA), lesion growth rate (LGR), spore production (SP), sporulating area (SA) and sporulation rate (SR) were determined as described elsewhere (Andrade-Piedra et al., 2005a;

Mizubuti and Fry, 1998; Suassuna et al., 2004). An aggressiveness index (A_i) for each isolate-host combination was calculated using the following equation: $A_i = \ln (LA \times SP \times 1/LP)$ (Montarry et al., 2007; Montarry et al., 2008).

4.5 LATEBLIGHT simulation model version LB2004 (Paper III and IV)

4.5.1 Assessing the adequacy of the simulation model LATEBLIGHT under Nicaraguan conditions (**Paper III**)

Five field experiments were implemented in two potato growing regions in northern Nicaragua and included two susceptible (Cal White and Granola) and one resistant (Jacqueline Lee) cultivars, the latter of which was recently introduced to Nicaragua. All three had a vegetative cycle lasting between 90 and 110 days under Nicaraguan conditions. Cal White and Granola were both known to be susceptible to *P. infestans,* while Jacqueline Lee was considered to be resistant to the US-8 strain in the United States (Douches et al., 2001; United States Potato Board, 2007). However, quantitative information on the level of resistance was not known for any of the cultivars. Each potato cultivar was planted separately. Fertilizers and non-experimental pesticide sprays were applied in accordance with the grower practices at each of the three locations.

The fungicide treatments consisted of three application intervals of the fungicide chlorothalonil (Knight 72 SC, 720 g a.i./L). Fungicide applications were initiated for a particular cultivar-location combination when percent emergence was at 50%. The first date of fungicide application was considered as the date of crop emergence and was used in the simulations. After the first fungicide application, plots were sprayed every 4, 7 or 14 days, depending on treatment. The fungicide chlorothalonil (Knight 72 SC) was applied at the recommended rate of 1.5 L/ha and ensuring a concentration of 2.52 g a.i./L of water. Plots without fungicide application were left as nontreated controls. Percent disease severity was estimated visually once a week starting 1 to 2 days after 50% plant emergence using a late blight standard area diagram (Anonymous, 1947), which had been modified by Fry (1977). Severity values from each epidemic (year-location-cultivar-treatment combination) were converted to the area under the disease progress curve (AUDPC) using the midpoint method (Campbell and Madden, 1990), and then to the relative AUDPC (RAUDPC) as described

earlier (Fry, 1978). To evaluate the level of susceptibility of the three cultivars to *P. infestans*, the RAUDPC was converted to susceptibility scale values as described by Yuen and Forbes (2009).

Simulations were performed with the LB2004 version of model LATEBLIGHT (Andrade-Piedra et al., 2005a). Initially, the parameters derived by Andrade-Piedra et al. (2005a) for the susceptible Peruvian cultivar Tomasa were evaluated in simulations with the epidemic data from Nicaragua for use with the two susceptible cultivars Cal White and Granola, but the results were not satisfactory. Therefore, modifications were made in the lesion growth rate (LGR) and the sporulation rate (SR) of the parameters from Tomasa. The modifications were made by visually fitting simulated and observed disease progress curves. The day of initiation of the epidemic and the number of initial lesions were determined by the method used by Andrade-Piedra et al. (2005b). It was evident from the disease severity data that cultivar Jacqueline Lee was protected by a major R gene and primarily had a hypersensitive resistance reaction. For this reason it was eliminated from the simulation process.

The original fungicide efficacy sub-model for LATEBLIGHT was developed and validated by Bruhn and Fry (Bruhn and Fry, 1981; Bruhn and Fry, 1982a; Bruhn and Fry, 1982b) specifically for chlorothalonil. In that model an average fungicide effect was calculated from the individual effects of the fungicide residues distributed among four levels in the canopy according to a gamma distribution (Bruhn and Fry, 1982a; Bruhn and Fry, 1982b).

In the present study, a simplified version in which there is no longer an effect of canopy level was employed. For our model, fungicide was assumed to be applied evenly on foliage. We believe this is justified in developing countries because we have observed that farmers using backpack sprayers tend to spray around the plant to achieve even coverage. The average level of deposition for foliage was estimated based on the concentration of the fungicide in the spray solution and a residue factor that indicated the amount of water that remains on the potato leaf surface when foliage is sprayed until run-off. A value of 0.0068 cm^3 water/cm^2 leaf was used for the residue factor (van Haren and Jansen, 1999). Details about the fungicide submodel structure are found in **Paper III.**

The adequacy of the model was investigated in several ways. Firstly, observed and simulated disease progress curves were compared graphically to evaluate the fit between observed and predicted data of disease progress with and without fungicides, and the overall pattern of disease severity relative to increasing fungicide application. Secondly, the efficacy of the

fungicide submodel was further evaluated by examining the number of cases when simulation falsely predicted control (false positive) or falsely predicted the absence of control (false negative). Control was arbitrarily defined to occur when final disease severity did not surpass 20% (Kromann et al., 2009). Thirdly, the simulator was also evaluated based on the deviations between observed and predicted AUDPC values. Deviations were compared to an envelope of acceptance test (EAT), the boundaries for which are calculated from the error of the observed values (Mitchell, 1997). The relationship between fungicide application and disease development measured by the AUDPC was explored with regression analysis, using the REG procedure of SAS (version 9.1; SAS Institute, Cary, NC). The quadratic regression equation provided the best fit for the relationship between the AUDPC and the fungicide spray interval. Cultivars were compared for RAUDPC values within each location-year combination using the least significant difference (LSD) test following an analysis of variance using PROC ANOVA of SAS v.9.1 (SAS Institute Inc., 2004). Normality of distributions and homogeneity of variances of experimental errors were tested as described by Quinn and Keough (2009).

4.5.2 Epidemiological significance of the quantitative relationship between host resistance and fungicide usage (Paper IV)

Simulation and field experiments were carried out with the following objectives: i) to determine the theoretical relationship between fungicide intensity and level of host plant resistance; and ii) to determine an optimum fungicide treatment strategy (timing and number of fungicide applications needed) to control late blight in accordance with the levels of host plant resistance of the cultivars and the prevailing geographical weather conditions of the experimental sites.

Nine levels of resistance were used with the simulation model LATEBLIGHT (version LB2004, Andrade-Piedra *et al.*, 2005b) to produce data points for assessment. Each resistance level represented a synthetic cultivar and was developed by altering four epidemiological parameters: latency period (LP), lesion growth rate (LGR) sporulation rate (SR) and infection efficiency (IE). During 2010, two simultaneous field trials were conducted at the Experimental Station Santa Catalina, Quito, Ecuador (International Potato Center, CIP, by its acronym in Spanish). In each trial, 12 potato cultivars with different degree of susceptibility to *P. infestans* were evaluated (Table 3). In both experiments, potato plants were grown in 3 m long × 4

m wide plots (four rows per plot and 10 plants per row, planted at 0.3 m spacing in the row). Each plot was separated from each other by a strip of 1 m oat. In the first field experiment, 12 treatments (12 potato genotypes) with three replicates were evaluated for their susceptibility to local population of *P. infestans*. In the second field experiment (hereafter referred to as "Trial II"), 72 treatments, resulting from the combination of six fungicide spray regimes and 12 potato genotypes were evaluated. More details on simulations and field experiments are described in **Paper IV**.

5 Results and Discussion

5.1 Sampling and isolation of *Phytophthora infestans* (Paper I and II)

Of the sampled isolates, 84% (209) isolates were isolated from blighted potato leaflets and 16% (39 isolates) were isolated from blighted tomato leaflets and fruits. All of the 248 collected isolates were tested for mating type, a subset of 132 isolates were used for microsatellite analysis and mtDNA haplotyping. Ninety-eight isolates (82 from potato and 16 from tomato) were used for fungicide sensitivity and used in virulence tests. Isolates for genotypic and phenotypic analyses were collected from 11 and 12 locations respectively (Table 1).

5.2 Genotypic and phenotypic characterization of *Phytophthora infestans* population from Nicaragua (**Paper I** and **II**)

5.2.1 Genotypic characterization

In the first study aimed to assess the genotypic diversity of Nicaraguan population of *P. infestans*, SSR genotyping using set of seven primers (4B, G11, Pi16, Pi70, D13, Pi63 and Pi04) revealed no polymorphism in 121 out of 132 isolates of *P. infestans* from Nicaragua. The only exception to this were two rare genotypes that showed one-step difference at the loci Pi16 and G11, respectively when compared to the commonly found genotype. Variations at the loci Pi16 and G11 were found in one and ten potato isolates respectively, representing 0.7% for the locus Pi16 and 7.6% for the locus G11 of the total isolates tested. These eleven potato isolates were collected from three different locations (El Arenal, Miraflor and Tisey; Table 1).

Otherwise, all the other isolates from potato and tomato belonged to a single multilocus genotype hereafter referred to as NI-1 genotype. This dominant genotype was heterozygous for almost all the analyzed loci (4B – 205/213; G11 – 132/156; D13 – 98/108; Pi63 – 148/157; and Pi04 – 166/170), except for Pi16 (176/176) and Pi70 (192/192) loci, which were found to be homozygous. Minor variants of this genotype were found with 176/174 at Pi16 (1 isolate) and 132/154 at G11 (10 isolates). Mitochondrial DNA (mtDNA) haplotyping revealed that all 132 isolates tested had the Ia haplotype. No evidence was found of population differentiation among potato and tomato isolates of *P. infestans* based on the SSR fingerprinting patterns and mtDNA haplotyping (**Paper I**).

In a second study, 72 isolates of *P. infestans* (53 from potato and 19 from tomato) from Nicaragua were further genotypically characterized using the same abovementioned SSR markers and mtDNA haplotyping. Five SSR multilocus genotypes among 72 isolates of *P. infestans* from Nicaragua were detected and all 72 isolates sampled from potato and tomato fields were of the Ia mtDNA haplotype and A2 mating type. The most predominant was the genotype NI-1, found in 63 out of 72 isolates and reaching a frequency of 87.5%. The N-1 genotype was common to 46 potato isolates and 17 tomato isolates. The frequency of the remaining four genotypes was very low (Figure 3). Variation in tomato isolates was found only in two isolates at loci 4B and Pi16 and in both cases they shared the same allele sizes with two potato isolates. In general, two kinds of variations were detected, namely, from heterozygosity to homozygosity at loci 4B and G11 and from homozygosity to heterozygosity at locus Pi16. The common trait of the five identified genotypes is that they belonged to the A2 mating type and had the Ia mtDNA haplotype. The 4B, G11 and Pi16 loci were the most variable loci, as they showed differences among tested isolates of *P. infestans* (Paper **II**).

Table 1. Origin, mating type, mitochondrial DNA haplotype and SSR fingerprinting pattern of *Phytophthora infestans* isolates collected from 2007 to 2010 in Northern Nicaragua.

Department	Location[a]	Crop	N-of-I[b]	Mating type	Haplotype[c]	SSR[d]
	El Jobo[P]	Potato	10	A2	nd[e]	nd
	La Laguna[G,P]	Potato	21	A2	Ia (13)	M[f] (13)
Estelí	La Tejera[G,P]	Potato	9	A2	Ia (3)	M (3)
	Miraflor[G,P]	Potato	39	A2	Ia (37)	M (34), V[g] (3)
	Sesteo[P]	Potato	23	A2	nd	nd
	Tisey[G,P]	Potato	34	A2	Ia (22)	M (20), V (2)
Sub-total	**6**		**136**	**136**	**75**	**75**
	Chagüite Grande	Tomato	12	A2	nd	nd
	El Canal[G]	Tomato	7	A2	Ia (7)	M (7)
	El Mojón[P]	Potato	3	A2	nd	nd
Jinotega	El Mojón[G]	Tomato	1	A2	Ia (1)	M (1)
	Las Colinas[P]	Tomato	4	A2	nd	nd
	La Galia[P]	Potato	10	A2	nd	nd
	La Parranda[G]	Potato	5	A2	Ia (5)	M (5)
	Tomatoya[P]	Tomato	5	A2	nd	nd
Subtotal	**7**		**47**	**47**	**13**	**13**
	Aranjuez[G]	Potato	1	A2	Ia (1)	M (1)
	El Arenal[G]	Potato	17	A2	Ia (17)	M (11), V (6)
Matagalpa	La Fundadora[G]	Potato	29	A2	Ia (18)	M (18)
	La Fundadora[G,P]	Tomato	10	A2	Ia (3)	M (3)
	Sitio Viejo[G]	Potato	5	A2	Ia (5)	M (5)
	Yucul[P]	Potato	3	A2	nd	nd
Sub-total	**5**		**65**	**65**	**44**	**44**
Total	**18**		**248**	**248**	**132**	**132**

[a]Isolates collected from locations marked with the letters G, P and GP, were used for genotypic (G) and phenotypic (P) analyses. In some cases the isolates were collected from the same location for both analyses (GP).
[b]Number of isolates collected from 2007 to 2010 in the main potato growing areas of northern Nicaragua.
[c]Mitochondrial DNA haplotype. In parenthesis is indicated the number of isolates that were tested.
[d]SSR = Simple sequence repeats (also known as microsatellites).
[e]nd = not determined or not included in the analysis.
[f]M = monomorphic for SSR markers. In parenthesis is indicated the number of isolates that were included in the analysis.
[g]V = variants, it means those isolates that showed one-step difference at loci G11 and Pi16. For instance in location Miraflor, 2 isolates had a one-step difference at locus G11 and 1 isolate showed one-step difference at locus Pi16. On the other hand, in other locations such as Tisey and El Arenal variation was observed only at locus G11.

Overall, 204 isolates of *P. infestans* (165 from potato and 39 from tomato) were analyzed using SSR markers and mtDNA haplotype determination in both studies (Table 2). It has been hypothesized that the first population of *P. infestans* present in Nicaragua belonged to the "old" single clonal lineage (US-1 genotype) of the A1 mating type and Ib mtDNA haplotype (Fry and Goodwin, 1997). This genotype probably arrived to Nicaragua in the early 1900s with a shipment of potato for consumption from United States. The Ia and IIb mtDNA haplotypes have been found in herbarium specimens from Nicaragua dating from 1954 and 1956 respectively (May and Ristaino, 2004). Our data suggests, however, that *P. infestans* populations have experienced a major shift since its first appearance in Nicaraguan potato fields.

Table 2. Simple sequence repeat (SSRs) multilocus genotypes detected in *Phytophthora infestans* isolates from Nicaragua collected from July 2007 to January 2010.

Gt[(a)]	NoI[(b)]	Host	Allele sizes[(c)] detected with seven SSR loci						
			4B	G11	Pi16	Pi70	D13	Pi63	Pi04
NI-1	195	P/T[(d)]	205	132	176	192	98	148	166
			213	156	176	192	108	157	170
NI-2	2	P/T	205	132	**176**	192	98	148	166
			213	156	**180**	192	108	157	170
NI-3	2	P	205	**156**	176	192	98	148	166
			213	**156**	176	192	108	157	170
NI-4	2	P/T	**213**	132	176	192	98	148	166
			213	156	176	192	108	157	170
NI-5	3	P	**213**	**156**	176	192	98	148	166
			213	**156**	176	192	108	157	170
Total	204								

[(a)]Gt: Genotypes found using seven SSR markers. The NI-1 genotype was the most predominant. The frequency of the other genotypes was very low.
[(b)]NoI: Number of isolates in a given genotype. The NI-1 genotype was common to 158 potato isolates and 37 tomato isolates, whereas 1 potato and 1 tomato isolate shared the same allele sizes and were grouped in the NI-2 and NI-4 genotypes.
[(c)]Allele sizes in bold are indicating where the variation was found. Allele sizes were adjusted to the sizes obtained by Lees et al. (2006).
[(d)]P/T: Potato or tomato host.

Genotypic diversity within populations of *P. infestans* from Nicaragua was expected due to the fact that potato seed is imported from the Netherlands, Canada, United States, and Guatemala. Contrary to this initial hypothesis, the *P. infestans* population from Nicaragua seems to belong to a single clonal lineage having the A2 mating type and the Ia mtDNA haplotype. Our results indicate that the Nicaraguan clonal lineage of *P. infestans* does not originate from seed imported from the Netherlands or other European sources, since the allele with the size 132 bp found at the locus G11 has not been recorded in European populations (D. Cooke, *personal communication*). This clonal lineage is not US-8, either, since that clonal lineage has a different genotype at these microsatellite loci (D. Cooke, *personal communication*).

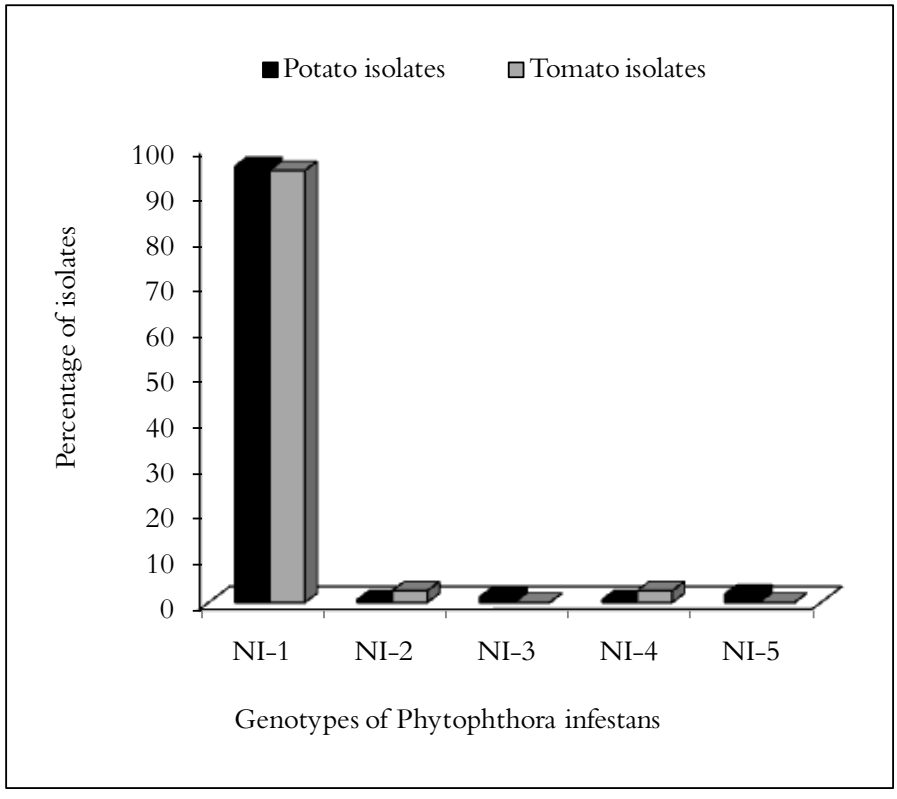

Figure 3. Genotypes of *Phytophthora infestans* detected using simple sequence repeat (SSRs) markers and the percentage of potato (n=165) and tomato (n=39) isolates found in each genotype.

The allele size 132 at G11 has been found in a *P. infestans* strain from Mexico (www.euroblight.net) and has been recorded from A1 tomato isolates from the United States such as US-11 and US-12 (D. Cooke, *personal communication*), suggesting a New World origin of the Nicaraguan population. In studies carried out in Venezuela using 4B and G11 SSR markers (Briceño et al., 2009) and Colombia using 4B and D13 markers (Vargas et al., 2009), a similar low genotypic diversity was found among the tested *P. infestans* isolates. In Central America, the mating type reported here for the Nicaraguan population of *P. infestans* is the opposite of that reported from neighboring countries. Transfer of agricultural products occurs over the borders of Nicaragua, Costa Rica and Honduras and one

might expect that isolates of *P. infestans* population from Costa Rica or Honduras have entered Nicaragua or vice versa. Nonetheless, there is no indication from the data presented here that such a transfer has taken place. The Costa Rican population of *P. infestans* belongs to two clonal lineages with the A1 mating type in potato and the A2 mating type in wild *Solanum* species (Gómez-Alpizar, 2004), while the *P. infestans* population from Honduras belongs to a clonal lineage having the A1 mating type (Forbes, 2004). We are not aware of any publications about the population structure of *P. infestans* from Guatemala and El Salvador.

The results from the multilocus analysis showed that Nicaraguan population of *P. infestans* is characteristically clonal in the distribution of genotypic variation, though new variants at very low frequencies were detected. This conclusion is also supported by the predominance of only one mating type (A2). The NI-1 single multilocus genotype dominated within the clonal lineage, comprising 158 potato isolates and 37 tomato isolates. This finding could indicate that there is neither host specificity nor genetic population differentiation between this group of potato and tomato isolates. Moreover, the movement of planting material (infected potato seed tubers and tomato seedlings) among and within production areas seems to be fostering a possible migration (genotype flow) of *P. infestans* strains between potato and tomato crops and consequently, preventing population differentiation and host specificity.

In Nicaragua, it is likely that the NI-1 genotype has completely replaced the "old" genotype (US-1) on both potato and tomato and consequently differentiation among potato and tomato isolates at genotypic level was not detected. No variation was found for mtDNA markers because only the Ia haplotype was detected. Hence, both studies (Paper **I** and **II**) confirmed that Ia is the dominant mtDNA haplotype in Nicaraguan population of *P. infestans*. Therefore, it is believed that the Ib mtDNA haplotype (US-1 genotype) has been completely replaced by the Ia haplotype. Moreover, it is likely that the Ia haplotype has also replaced the IIb haplotype which was found in herbarium specimen from Nicaragua dating from 1956 (May and Ristaino, 2004).

So far, the NI-1 is still the most widely distributed and dominant genotype within Nicaraguan clonal lineage of *P. infestans*. It is also known that this genotype is formed by non-host specific potato and tomato strains, which are resistant to metalaxyl and has a complex race structure (**Paper I**) However, the occurrence of new variants could pose a greater threat for potato and tomato crops in Nicaragua, especially, if these variants are equally or more pathogenic and more ecologically adapted than the

predominant NI-1 genotype. Therefore, more extensive sampling at the sites from which isolates were recovered and genotyping of these new variants would be required to track the movement and diversification of these variants. Substantial shifts in *P. infestans* populations have occurred in UK for instance, where in just four years the prevalence of genotype 13_A2 or "Blue 13", rose to 79% of late blight outbreaks. The "Blue 13" genotype was first detected in the Netherlands in 2004 (Cooke et al., 2008). A similar situation has been experienced in the United States, where over a period of five years the US-8 genotype became the most widely distributed, dominant and troublesome genotype (Fry and Goodwin, 1997a). Nicaragua annually import potato seed from the Netherlands, therefore the occurrence of the "Blue 13" genotype in the Netherlands could have very serious epidemiological implications for potato production in Nicaragua regardless of the fact that the "Blue 13" genotype appears to be better adapted to cooler temperatures.

5.2.2 Phenotypic characterization

All of the isolates collected from different locations in Northern Nicaragua were characterized as A2 mating type (Table 3). Ninety-six isolates (including both potato and tomato isolates) were resistant to metalaxyl (98%), 1 intermediate (1%) and 1 sensitive (1%) to metalaxyl (Table 3). The latter two were potato isolates. Fifty-three isolates (54%) were able to sporulate in propamocarb-HCl at 10 mg/L, 27 isolates (28%) sporulated in propamocarb-HCl at 100 mg/L. No isolate was able to sporulate at 1000 mg/L (Figure 4).

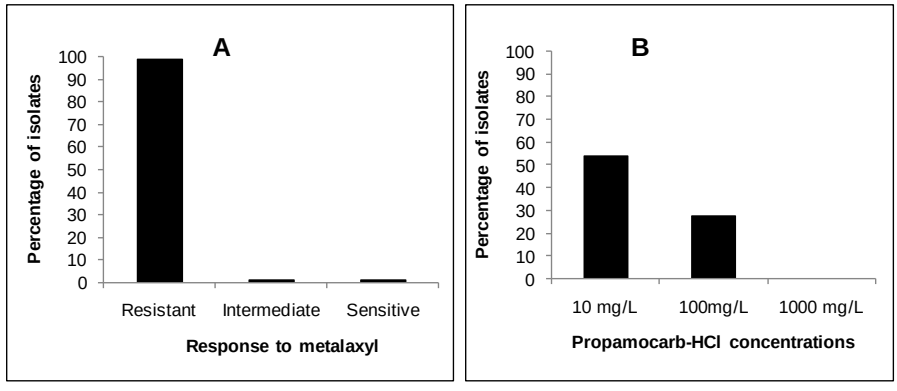

Figure 4. Response of *Phytophthora infestans* isolates from Nicaragua (sampled in 2008-2009) to phenylamide fungicide metalaxyl (**A**) and propamocarb hydrochloride (**B**). Samples were taken from tomato and potato fields in different locations in Northern Nicaragua.

The results from the virulence testing showed a high variation among isolates of *P. infestans* from Nicaragua. Among the 82 potato isolates 31 races were found. The most frequent race in the potato isolates was R1.2.3.4.5.6.7.10.11 (14 isolates), followed by R1.2.3.4.6.7.10.11 (9 isolates), R1.3.4.5.7.10.11 (7 isolates), R1.3.4.7.11 (6 isolates) and R1.3.4.7 (5 isolates). Fifteen races were represented by a unique isolate, whereas the remaining number of races (12) was represented by two and four isolates (Table 3).

Among the 16 tomato isolates 11 races were identified, that is, almost one race per isolate tested. The most frequent races found in tomato isolates were R1.3.4.7. (3 isolates) and R1.3.4.7.11 (3 isolates) followed by R1.3.4 (2 isolates). The remaining number of tomato races was represented by a unique isolate (Table 3). Both potato and tomato isolates overcame the resistance gene R1 at the same proportion (88%). The remaining resistance genes were overcome at different proportions depending on the isolate tested. Only one potato isolate collected during 2008 was able to overcome resistance gene R9. No isolate was able to overcome the resistance gene R8 (Figure 5).

The number of virulence factors in each isolate ranged from 2 to 9 in both potato or tomato isolates. Among the potato isolates, seventeen (grouped in four races) were found to have eight virulence factors. The *Ci* and *Cp* were 6.4 and 5.5 respectively for potato isolates, while for tomato isolates *Ci* and *Cp* were 5.0 and 5.4 respectively. The *Ci* was higher than

Cp in potato isolates, indicating that complex races predominate within potato populations of *P. infestans* from Nicaragua. The *t-test* procedure showed no significant differences between potato and tomato isolates for the *Ci* and *Cp* values (data not shown). Overall, the phenotypic analysis also revealed no population differentiation among potato and tomato isolates of *P. infestans* from Nicaragua.

Potato and tomato isolates of *P. infestans* from Nicaragua were tested for their aggressiveness toward potato and tomato detached leaflets in cross-inoculation experiments. A significant effect among potato isolates with regard to IP ($P=0.04$), LA ($P=0.05$) and LGR ($P=0.01$) was found. Potato isolates induced necrotic spots in tomato leaflets earlier than on potato leaflets, produced larger LA in potato leaflets and the LGR was greater in potato leaflets than in tomato ones. Highly significant differences among potato isolates for LP ($P<0.0001$), SP ($P<0.0001$), SR ($P<0.0001$) and AI ($P<0.003$) were found. Potato isolates had a shorter LP, produced more sporangia (SP), had a greater SR and were more aggressive (AI) on tomato leaflets than in potato ones.

Potato isolates were not statistically different regarding SA ($P=0.73$). No significant differences for IP ($P=0.75$), LA ($P=0.95$) and LGR ($P=0.33$) among tomato isolates were found. Highly significant effects among tomato isolates for LP ($P<0.0001$), SP ($P<0.0001$), SA ($P<0.006$) and AI ($P<0.0001$) were detected. The mean values for SR among tomato isolates were statistically significant ($P=0.05$). Tomato isolates had a shorter LP, produced more sporangia (SP), had a greater SA and were more aggressive on tomato leaflets than on potato ones. Potato and tomato isolates both had a shorter LP, higher SP and were more aggressive on tomato leaflets compared to potato leaflets (Table 4).

Although the Nicaraguan population of *P. infestans* was found to be dominated by a single clonal lineage (NI-1 genotype), it contained a high variability with regards to virulence spectra and fungicide insensitivity. This is in agreement with the results from a similar study conducted in Northern China, in which low genotypic diversity was observed, while the virulence spectra turned out to be highly variable. Moreover, they also found that some of the tested isolates were virulent to all R-genes (Guo et al., 2009). However, unlike the Chinese population of *P. infestans*, the Nicaraguan one could not overcome all of the R-genes present in the traditional differential set of potato clones.

Table 3. Race structure and response to metalaxyl-M of *Phytophthora infestans* isolates collected from potato and tomato plants during 2008 and 2009 in the main potato and tomato growing areas of Nicaragua.

Locations[a]	Races[b]	RM[c]	N-of-I[d]
LC	$R2.3^{T}$	R (1)	1
LG	$R2.11^{P}$	R (1)	1
LL	$R3.7^{P}$	R (1)	1
MF	$R3.11^{P}$	R (1)	1
LF, TM	$R1.3.4^{T}$	R (2)	2
EJ, LL	$R1.3.7^{P}$	R (2)	2
EJ	$R3.4.7^{P}$	R (1)	1
LG, MF	$R3.4.11^{P}$	R (2)	2
ST	$R3.7.11^{P}$	R (1)	1
LF, LL, ST, TM	$R1.3.4.7^{P(5),T(3)}$	R (8)	8
LF, ST, YC	$R1.3.4.11^{P(2),T(1)}$	R (3)	3
TM	$R2.3.7.11^{T}$	R (1)	1
ST	$R3.4.7.11^{P}$	I (1)	1
LL	$R1.2.3.4.7^{P}$	R (1)	1
LT	$R1.3.4.5.11^{P}$	R (1)	1
LL	$R1.3.4.5.7^{P}$	R (1)	1
EJ, LC, LF, LL, LT, ST, TM	$R1.3.4.7.11^{P(6),T(3)}$	R (9)	9
ST	$R2.3.4.6.11^{P}$	R (1)	1
LG	$R3.4.7.10.11^{P}$	R (1)	1
LF	$R1.2.3.4.6.7^{T}$	R (1)	1
LT, ST	$R1.2.3.4.7.11^{P}$	R (2)	2
TM	$R1.3.4.5.7.11^{T}$	R (1)	1
TY	$R1.3.4.5.10.11^{P}$	R (2)	2
ST	$R1.3.4.6.7.11^{P}$	R (1)	1
MF, TY	$R1.3.4.7.10.11^{P}$	R (4)	4
ST	$R1.2.3.4.5.6.11^{P}$	R (1)	1
LG, LT	$R1.2.3.4.6.7.11^{P}$	R (2)	2
LG, TY	$R1.2.3.4.7.10.11^{P}$	R (2)	2
EJ, ST, TY	$R1.3.4.5.7.10.11^{P}$	R (6) S (1)	7
TY	$R1.3.4.6.7.10.11^{P}$	R (1)	1
ST, TY	$R1.2.3.4.5.6.7.11^{P}$	R (4)	4
LT, ST	$R1.2.3.4.5.6.10.11^{P}$	R (2)	2
LF	$R1.2.3.4.5.7.10.11^{T}$	R (1)	1
EJ, LF LG, LT, MF, ST, TY	$R1.2.3.4.6.7.10.11^{P(9),T(1)}$	R (10)	10
LT, ST	$R1.3.4.5.6.7.10.11^{P}$	R (2)	2
EM	$R1.2.3.4.5.6.7.9.11^{P}$	R (1)	1
EJ, LF, LT, MF, ST, TY	$R1.2.3.4.5.6.7.10.11^{P(14),T(1)}$	R (15)	15
	Total	98	98

[a]EJ = El Jobo; EM = El Mojón; LC = Las Colinas; LF = La Fundadora; LG = La Galia; LL = La Laguna; LT = La Tejera; MF = Miraflor; ST = Sesteo; TM = Tomatoya; TY = Tisey; YC = Yucul.

[b]The host origin of the isolates belonging to each race is indicated by the letter P (potato plants) and T (tomato plants). In parenthesis is indicated the number of isolates sampled from each host plant and used for virulence testing.

[c]RM = Response to metalaxyl-M; R = resistant; I = intermediate resistant; S = susceptible. In parenthesis is indicated the number of isolates in each category. The susceptible isolate was collected in location Tisey.

[d]N-of-I = number of potato and tomato isolates used in fungicide sensitivity tests and in virulence testing using the potato differential set of R-genes (R1 to R11).

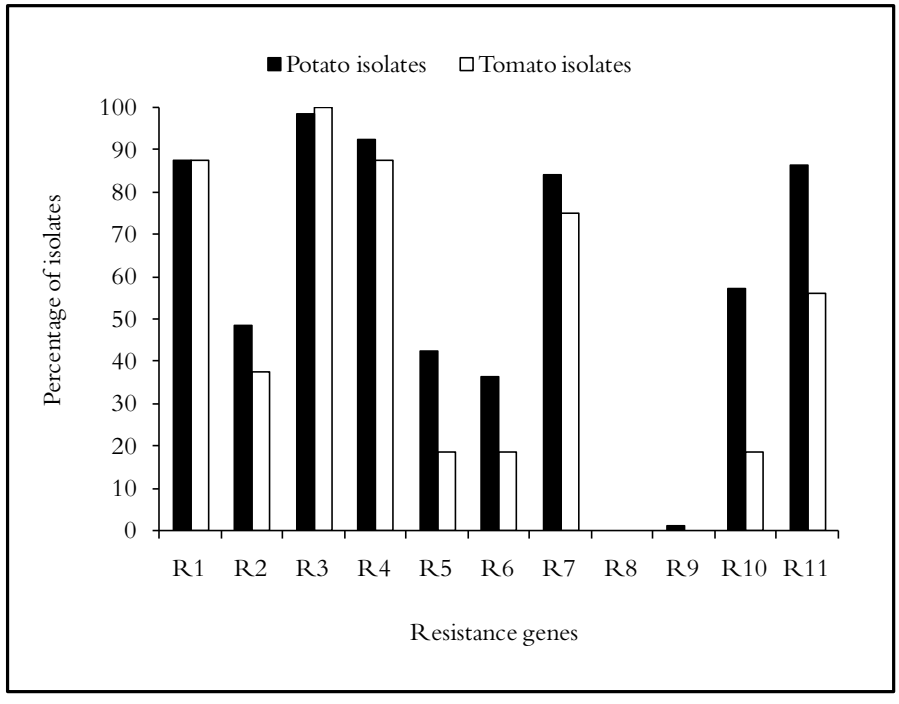

Figure 5. Percentages of *Phytophthora infestans* isolates from Nicaragua overcoming resistances genes (R-genes, R1 to R11). These isolates were collected during 2008 and 2009 from commercial potato and tomato fields.

Table 4. Least square means (LSMEANS) values of the aggressiveness components resulting from cross-inoculation tests with potato and tomato isolates of *Phytophthora infestans* in potato and tomato leaflets.

AC[a]	Potato isolates			Tomato isolates		
	Leaflet		*P* > F	Leaflet		*P* > F
	Potato	Tomato		Potato	Tomato	
IP[b]	2.01 (48)	1.90 (46)	0.04	1.81 (43)	1.78 (43)	0.75
LP[c]	3.61 (87)	2.78 (67)	0.0001	4.54 (109)	2.75 (66)	0.0001
LA[d]	1275	1128	0.05	1071	1076	0.95
LGR[e]	4.88 (10^{-3})	4.52 (10^{-3})	0.01	4.06 (10^{-3})	4.24 (10^{-3})	0.33
SP[f]	23413	45979	0.0001	11499	39057	0.0001
SA[g]	1047	1071	0.73	766	1015	0.006
SR[h]	2.5 (10^7)	3.9 (10^7)	0.0001	2.9 (10^7)	3.7 (10^7)	0.05
AI[i]	12.4	13.0	0.003	11.3	12.9	0.0001

[a]Aggressiveness components

[b]IP = Incubation period [time (days) after inoculation when necrotic spots appeared; in parenthesis is indicated the IP in hours];

[c]LP = Latency period [time (days) after inoculation when sporangia appeared; in parenthesis is indicated the LP in hours];

[d]LA = Lesion area (mm^2) including the sporulating annulus (divide by 10^6 to convert it to square meters);

[e]LGR = Lesion growth rate, measured in meters per day;

[f]SP = Spore production calculated by multiplying the sporangia concentration by the volume of a preservative solution [(0.04 M copper sulfate, 0.2 M sodium acetate/acetic acid, pH 5.4); Mizubuti and Fry, 1998)].

[g]SA = Sporulating area (mm^2), which is the difference between LA and the area before the LP (hours); divide by 10^6 to convert it to square meters.

[h]SR = Sporulation rate (sporangia per square meter per day), which is calculated using the equation SR=SP/SA;

[i]AI = Index of aggressiveness, calculated by the formula AI=ln(LA x SP x 1/LP) according to Montarry et al. (2007).

The most complex and most common races of *P. infestans* populations from Nicaragua overcame eight and nine resistance genes, respectively. Race complex structure and high virulence diversity have been detected in other parts of the world (Barquero et al., 2005; Deahl et al., 2003; Pérez et al., 2001). Race complexity observed in *P. infestans* isolates from Nicaragua can have arisen as a result of the selection pressure imposed by potato cultivars carrying different resistance genes (R-genes). The potato cultivar Santé, which is the preferred cultivar among Nicaraguan potato growers, is known to carry the resistance genes R1 and R10 (Flier et al., 2007). The appearance of new races is related to mutations in avirulence (*Avr*) genes encoding effector proteins in such a way that the effectors are not able to be recognized by the R protein (Guo et al., 2009). Since the *Avr* genes are positioned in a hypervariable fast evolving part of the genome (Jiang et al., 2008) this could explain the high virulence diversity in isolates with the same SSR multilocus genotype.

Metalaxyl-containing products are currently rarely applied in Nicaragua, if at all. In spite of this, a high percentage of the tested isolates were shown to be resistant to metalaxyl. In other places around the world, a high percentage of *P. infestans* isolates resistant to metalaxyl has also been found (Chen et al., 2009; Deahl et al., 2003; Gómez-Alpizar, 2004; Pérez et al.,

2009). Resistance to phenylamide fungicides, such as metalaxyl, can naturally occur as a result of random mutation (Dagget et al., 1993; Gisi and Cohen, 1996). Nonetheless, pre-existing resistant individuals increase in frequency as a result of the selection pressure imposed by fungicide application (Gisi and Cohen, 1996; Grünwald et al., 2006).

In Nicaragua, the appearance and increase of metalaxyl-resistant isolates probably occurred in the 1980s and early 1990s when the potato production areas were increased and metalaxyl-based fungicides were used frequently and indiscriminately. Therefore, this could lead to a directional selection toward resistance which persists in the current clonal Nicaraguan population of *P. infestans*. This could also result in a reduction in genotypic diversity as has been reported in other studies (Grünwald et al., 2006). In spite of the infrequent use of metalaxyl, the phenotypic trait of metalaxyl resistance remains at a high frequency in the Nicaraguan population of *P. infestans*. This may be explained by a clonal population, which will retain unnecessary traits longer than a sexual reproducing population.

The sensitivity of Nicaraguan isolates of *P. infestans* against propamocarb hydrochloride was also tested. This fungicide is used by Nicaraguan potato growers formulated alone or in mixture with other fungicides with different mode of action. As was pointed out earlier, no evidence of resistance to propamocarb-HCl was found when these isolates were exposed to the highest concentration (1000 mg/L) of the fungicide. Potato growers in Nicaragua apply propamocarb-HCl at a rate of 1083 mg/L active ingredient (a.i.) when formulated alone and 564 mg/L a.i. when formulated as a mixture with another fungicide. Although 28% of the tested isolates were able to sporulate in propamocarb-HCl at a concentration of 100 mg/L a.i., the lower fungicide rate applied by potato growers in the field is five times greater than that in which sporulation was detected. There are some reports from other parts of the world of *P. infestans* isolates resistant to propamocarb-HCl (Lehtinen et al., 2008; Möller et al., 2009).

A study was also undertaken with the objective to establish whether there are differences in aggressiveness among *P. infestans* potato and tomato isolates through reciprocal aggressiveness tests. Some observations done in this study would support the hypothesis that tomato is a better host than potato due to the following: i) the time elapsed between the end of the IP (appearance of small necrotic spots) and the beginning of the LP (appearance of sporangia) was shorter on tomato leaflets than on potato ones, that is, the LP was shorter than IP, showing that potato isolates displayed a biotrophic colonization phase on tomato leaflets as has been reported in other studies (Smart et al., 2003; Suassuna et al., 2004; Vega-Sánchez et al., 2000); ii) the

LP was shorter on tomato leaflets than on potato ones; iii) the LA was greater on tomato leaflets, indicating that disease intensity is expected to be higher on tomato; iv) The SP was 1.9 times greater on tomato leaflets than on potato ones; v) the aggressiveness index was greater on tomato leaflets than on potato ones, and was almost the same as the aggressiveness index of the tomato isolates on tomato leaflets, suggesting that potato and tomato isolates are equally aggressive on tomato.

Contrary to our initial hypothesis, tomato isolates performed better on tomato leaflets than on potato ones. This finding could indicate host-specificity of tomato isolates toward tomato. In general, tomato isolates were more aggressive on its host of origin, whereas potato isolates were more aggressive on the alternative host. Although SSR fingerprinting and mtDNA haplotyping showed no differentiation between potato and tomato isolates, aggressiveness tests revealed that tomato isolates showed a general, but not exclusively, host-specificity and were more aggressive on tomato. In contrast, potato isolates showed host-preference toward tomato detached leaflets and were more aggressive on them.

5.3 LATEBLIGHT simulation model version LB2004 (Paper III and IV)

5.3.1 Assessing the adequacy of the simulation model LATEBLIGHT under Nicaraguan conditions (**Paper III**)

Disease onset varied across experiments and between the two susceptible cultivars, with a range of initiation times from 8 to 20 days after emergence (Table 5). These weather parameters characterized highly disease-conducive conditions for susceptible cultivars CalWhite and Granola. Late blight was detected in cultivar Jacqueline Lee only at negligible levels in all plots (including nontreated plots) at the end of the growing season in the three locations (data not shown).

The simulation model generally predicted high disease severity in the absence of fungicide application, and demonstrated a decrease in the disease progress curves with additional fungicide applications, approximately similar to the observed data. Based on this visual assessment, we concluded that the epidemic model and fungicide submodel were generally applicable to Nicaraguan conditions. Moreover, the model predicted that fungicide as applied would not be sufficient for adequate disease control. However, the

model did not perform well based on the more stringent EAT test, as only 15 of 40 epidemics were within the boundaries of the envelope.

Based on mean observed RAUDPC for nontreated plots, Granola was only slightly less susceptible than CalWhite and in only two experiments the difference was significant ($\alpha = 0.05$). For Jacqueline Lee, calculation of scale values was consistent with a hypothesis of hypersensitive-based resistance, as a value of 0 was derived in each experiment. Using visual assessment of disease progress curves and a 20% cut-off for final disease severity, no fungicide treatments, including fungicide application at four day intervals, were found to be adequate for managing the disease in the susceptible cultivars in any of the experiments.

Table 5. Geographic location, disease onset, temperature and humid hours of field trials done in northern Nicaragua to test host resistance in potato to *Phytophthora infestans* and efficacy of different fungicide application frequencies.

Location/Year	Disease onset (days after emergence)			Weather variables	
	Cal White	Granola	Jacqueline Lee	T[a]	H_hr[b]
Arenal 2007[c]	11	11	21	17.1	17.3
Miraflor 2007[d]	8	11	30	16.2	18.9
Tisey 2007[e]	17	20	30	16.3	16.2
Miraflor 2008	8	12	26	19.1	19.5
Tisey 2008	10	17	25	18.1	19.9
Average	11	14	26		

[a] T = mean daily air temperature.
[b] H_hr = hours per day of relative humidity >85%.
[c] Arenal 2007: latitude 13°02'13" N, longitude 85°55'03" W and 1380 meters above sea level (masl).
[d] Miraflor 2007-2008: latitude 13°15'59" N, longitude 86°16'44" W and 1390 masl.
[e] Tisey 2007-2008: latitude 12°59'36" N, longitude 86°22'07" W and 1450 masl.

There was a clear and generally linear reduction in the observed AUDPC relative to the number of applications up through the seven day intervals, which was equal to nine applications. The additional six applications of the four day intervals did not appear to give the same relative decrease in the AUDPC.

The simulation model was generally predictive, but the degree of predictability depended on the type of evaluation measure used. Based simply on its ability to predict fungicide efficacy it would appear to work reasonably well; and therefore, we conclude that it is adequate for exploring general aspects of fungicide efficacy under Nicaraguan conditions. As a more specific case of this type of predictability, the model successfully indicated that in general, frequent sprays of chlorothalonil would not be sufficient to control disease in susceptible cultivars under the prevailing environmental conditions.

When model performance was assessed using the EAT, it did not perform as well as in a previously published study. Andrade-Piedra et al. (2005b) found that over 75% of epidemics they studied in Peru fell within the EAT, which was a higher percentage than identified in this study. However, it is important to note that the earlier study only focused on nontreated plots and did not involve the fungicide sub-model. Tedeschi (33) indicated that model appropriateness should depend on its primary use. We intended to use the model to explore fungicide efficacy, and for that purpose the model was adequate.

In spite of general predictive capacity, the model also had systematic bias, as evidenced by the regression of simulated AUDPCs on observed. The slope of the line was greater than one in each year; the model tended to under-predict at low disease levels and over-predict at higher levels. A previous study indicated that the epidemic model in LB2004 is generally accurate over a wide range of environments with variable amounts of late blight severity (Andrade-Piedra et al., 2005c), thus we hypothesize that the bias is most likely occurring in the fungicide sub-model, which has been used for the first time in this study; however, some systematic error in the epidemic model may have also occurred.

In our study, simultaneous experiments with systemic and contact fungicides to compare their respective effectiveness were not conducted. Nevertheless, it would seem that use of more effective systemic or translaminar fungicides would be a logical alternative for improving management of late blight in this system, as in other systems (Kromann et al., 2009; Stein and Kirk, 2003). This would appear to be true for the Central American and Andean highlands, but may also be true for other tropical highland locations. The 20% cut-off we used for determining fungicide efficacy was also used by Kromann et al. (2009), however, it was the "best guess" used for comparative purposes and is not derived from experimental data and does not constitute an economic or action threshold.

In developing countries, including Nicaragua, potato growers rely on the readily available contact fungicides chlorothalonil and mancozeb. Nonetheless, our study demonstrated a lack of adequate fungicide protection with chlorothalonil on cultivars Cal White and Granola, which indicates the need for cultivars with higher levels of durable resistance. In their absence, farmers should consider more effective fungicide applications, involving either higher dosages or different chemistries, including systemic and translaminar products.

In addition to lack of control in this study, chlorothalonil and mancozeb have been identified as probable carcinogens (Stein and Kirk, 2003).

Therefore, the LB2004 simulation model should be modified to simulate the effects of other fungicides that pose a lower health and/or environmental risk, and are potentially more effective, including those with systemic or translaminar properties.

5.3.2 Epidemiological significance of the quantitative relationship between host resistance and fungicide usage (**Paper IV**)

The environmental conditions were diverse in the three locations chosen to run the simulations. In Arenal-2007 (Nicaragua) the weather conditions were conducive for late blight development, while in CIP-2010 (Ecuador) and Huancayo-2000 (Peru) the weather conditions were less conducive for late blight epidemics, as evidenced by lower average temperatures and lower hours of relative humidity above 85% (Table 6).

The number of spray intervals that would keep the disease threshold below a given level depended on the epidemic duration and the local weather conditions. In Arenal-2007, CIP-2010 and Huancayo-2000, the number of spray intervals needed were 26, 40 and 40 respectively, in order to keep the final disease level below a pre-determined disease threshold. According to the results from the simulation, in conditions less favorable for late blight development, potatoes with even high susceptibility levels (as well as more resistant ones) could be grown. As conditions became more favorable for disease development, only the less susceptible cultivars could be grown without exceeding the pre-determined disease threshold. In Huancayo-2000, for instance, one could control the disease with at most 20% final severity with any resistance level even with the 21 day interval. In CIP-2010, many resistance levels could be grown with 21 day spray interval for both the 15 and 20% final disease severity threshold.

On the other hand, in Arenal-2007, late blight control can be attained spraying every two weeks, but using cultivars with a scale value in between 1 and 2 and disease thresholds ranging from 10% to 20% final severity. A curvilinear relationship between spray interval and resistance was evident, but a plot of the logarithm of the spray interval versus cultivar susceptibility showed a more linear relationship (Figure 6).

In the field experiment, significant differences ($P = 0.04356$) among evaluated potato genotypes with regard to susceptibility to *P. infestans* were found. According to the RAUDPC and scale values, the less susceptible potato genotypes to *P. infestans* were Pastusa Suprema and I-Estela, whereas the most susceptible genotypes were Cecilia and I-Gabriela. The tested

potato genotypes were grouped in five categories based on the scale values (Table 7).

Table 6. Characteristics of three locations used in the study

Location, year, country	Altitude[a]	Latitude	Longitude	T[b]	H_hr[c]
Arenal, 2007, Nicaragua	1380	13°02'13"N	85°55'03"W	17.1	17.3
CIP, 2010, Ecuador[d]	3058	00°22'S	78°33'W	12.0	12.7
Huancayo, 2000, Perú	3300	12°1'45"S	75°14'0"W	12.0	10.6

[a]Meters above sea level.
[b]T = mean daily air temperature.
[c]H_hr = hours per day of relative humidity >85%. This threshold of relative humidity was used because the sensor was located above the potato canopy.
[d]International Potato Center (CIP), Quito, Ecuador.

Table 7. Susceptibility of 12 potato genotypes to *P. infestans*, measured in field experiment implemented at the Experimental Station Santa Catalina, Quito, Ecuador (International Potato Center, CIP, by its acronym in Spanish) in February 2010.

Potato genotypes	**RAUDPC[1]**	**Scale[2]**
Pastusa Suprema	0.34 a	4
I-Estela	0.38 ab	4
I-Natividad	0.41 ab	5
Nova	0.45 abc	5
I-Fripapa	0.49 bc	6
Carolina	0.49 bc	6
Unica	0.50 bc	6
Betina	0.55 cd	6
Roja Nariño	0.57 cde	7
Superchola	0.63 def	7
I-Gabriela	0.70 ef	8
Cecilia	0.73 f	8

[1]RAUDPC = Relative area under the disease progress curve corresponding to each of the potato genotypes evaluated in Ecuador in 2010. Values within the column followed by the same letter are not significantly different at $P = 0.05$ (Tukey´s HSD).
[2]Values corresponding to the scale proposed by Yuen and Forbes (2009) to evaluate the susceptibility of potato genotypes.

Simulated and real (field) spray intervals as a function of potato susceptibility were also plotted (Figure 7). In simulations with the weather data CIP-2010, the fungicide need was fixed at 21 day applications intervals, but with 15% and 20% disease thresholds. Short application intervals (4 to 8) were not needed. Application intervals of 10 to 21 days were found more frequently even with very high scale values of susceptibility. The curvilinealirity of the relationship between fungicide application intervals and the scale values of susceptibility of potato cultivars was much clearer in field experiments. As

the susceptibility levels of the potato cultivars moved to the left in the scale, the fungicide application intervals increased.

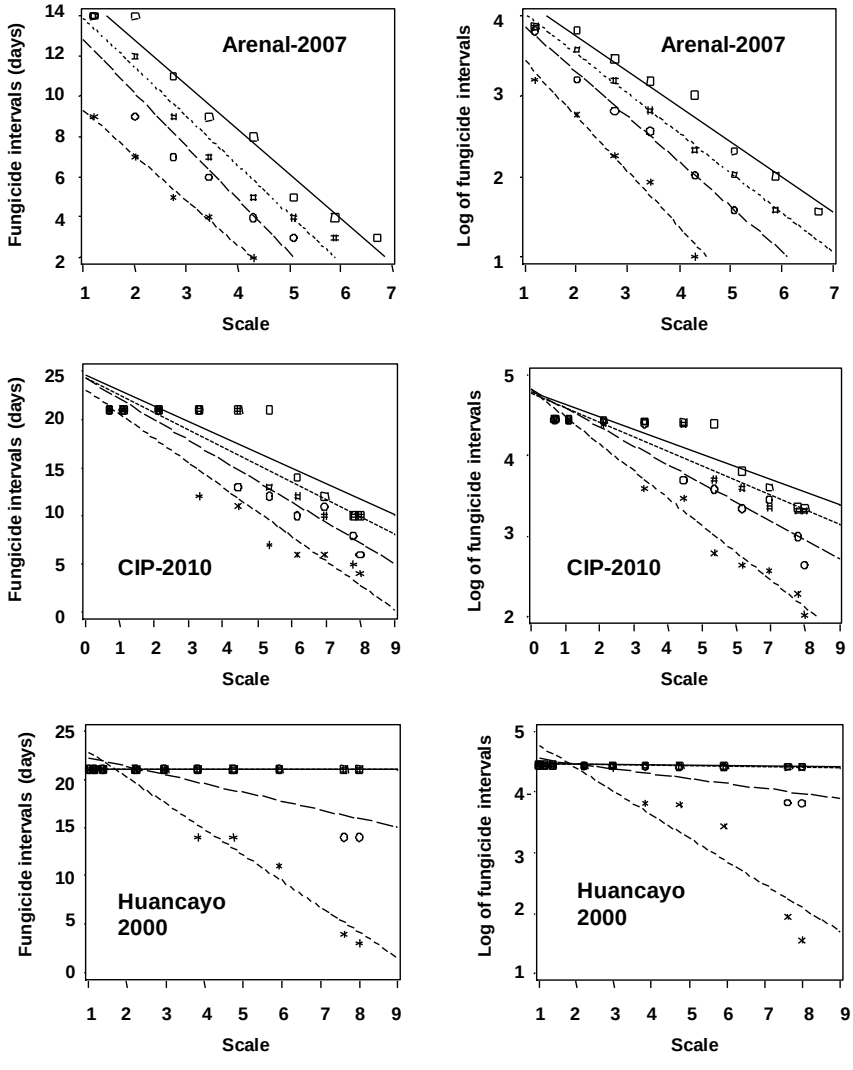

Figure 6. Relationship between potato susceptibility (scale values) and fungicide application frequency (intervals) at 5% (⋆), 10% (o), 15% (#) and 20% (□) final disease severity (thresholds). The simulations were performed using the weather data from three different locations and three different years: Arenal-2007 (Nicaragua), CIP-2010 (Ecuador) and Huancayo-2000 (Peru).

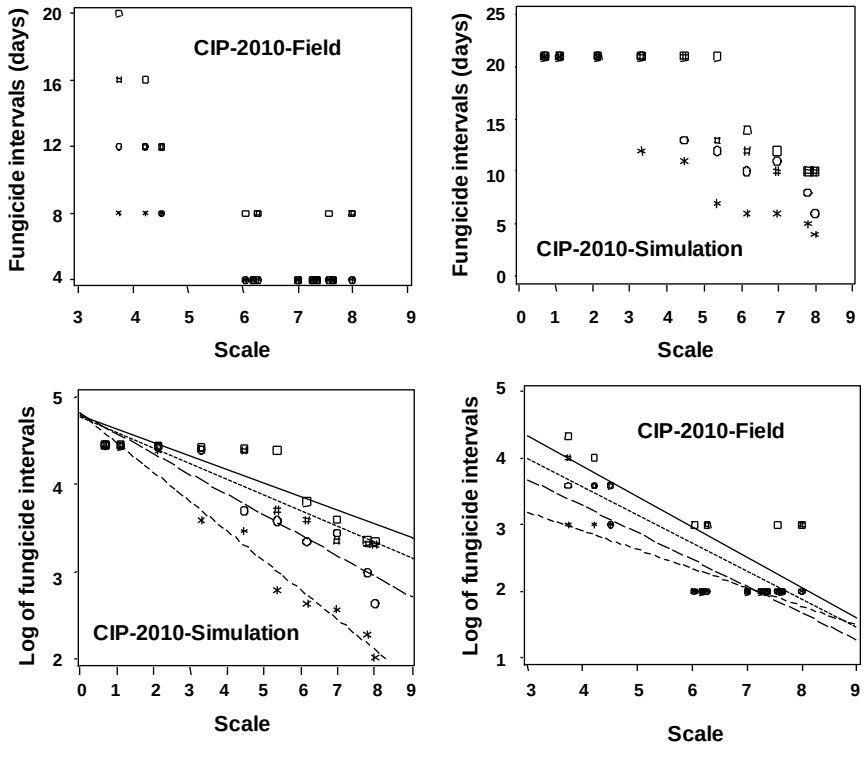

Figure 7. Comparison of simulated and real (field) fungicide application intervals as a function of susceptibility of synthetic and real potato cultivars. The field experiments were implemented at the Experimental Station Santa Catalina, Quito, Ecuador. Four disease thresholds, expressed as final severity were used to make comparisons between simulated and field experiments: 5% (⋆), 10% (o), 15% (#) and 20% (□).

Dependence on the use of fungicides to control late blight and its accompanying yield losses could be reduced if more resistant cultivars and an appropriate deployment of host resistance were used (Garret et al., 2001). This positive effect of host resistance on fungicide efficacy has been reported (Fry, 1978). In the present study, the simulation model LATEBLIGHT demonstrated a quantitative trend between host resistance and fungicide requirements. The marginal effect of susceptibility on changing the need for fungicides was low for potato cultivars with high susceptibility, and higher for potato cultivars with low susceptibility. Thus, for potato breeding programs, greater benefit can be expected by reducing

susceptibility to below the midpoint on the scale. The pattern described above was evident with three different weather data sets representing different levels of disease pressure. Attempts were made to validate this relationship with field data. Similar trends regarding the nature of the relationship between susceptibility and spray interval were evident in the data from the field trial, but discrepancies between the observed and simulated epidemics are present.

The weather-based nature of potato late blight (Forbes and Simon, 2007) can be seen in the comparison of epidemics from three different locations: Arenal-2007 (Nicaragua), CIP-2010 (Ecuador) and Huancayo-2000 (Peru). Simulation was consistent with the hypothesis that colder and drier conditions such as those observed in Huancayo-2000, are less conducive to late blight development. This is a confirmation of indigenous knowledge as historically farmers in the Andes have tended to put their most susceptible cultivars in higher locations (Kromann et al, 2009).

In contrast, Arenal-2007 seemed to provide more optimal conditions for late blight epidemics. Thus, under Nicaraguan conditions control of late blight could be achieved spraying weekly and using potato cultivars with scale values in between 2 and 4 at any disease threshold. Moreover, almost immune potato cultivars should be used under Nicaraguan conditions if one would like to spray every two weeks, but extremely susceptible cultivars will need sprays quite often, as was seen in other studies (**Paper III**, this thesis).

The final disease thresholds used to judge the adequacy of fungicide use (5 to 20 percent) were completely arbitrary. What could be tolerated in actual practice would depend on a number of factors, such as the effect on crop yield as well the effect on crop quality. Under conditions where the crop is sold immediately, as is common in many highland tropical situations, latent infections of *P. infestans* may not be as critical as they would be in situations where the tubers are stored during the winter and the latent infections lead to visible damage.

While plant breeders often refer to characteristics such as latency period, lesion growth rate, spore production, and infection efficiency as components of resistance, plant disease epidemiologists also use them to describe pathogen characteristics, calling them components of aggressiveness. They are, however, both the result of the interaction between a specific pathogen genotype, or isolate, and a specific potato cultivar. A more neutral term would be to refer to them as epidemiological parameters. These were manipulated in order to generate the synthetic resistant cultivars used in this simulation, and they were all reduced

simultaneously to produce the different levels of resistance. While this is what has been observed in some studies, there are also sources of resistance that reduce one of the epidemiological parameters more than others. The effect of the individual epidemiological components on the intensity of fungicide use was not studied in these simulations, and some could be more effective than others.

In general, the simulation model LATEBLIGHT (version LB2004) showed trends, which could be used, although with cautions, to adjust the fungicide application intervals in accordance with the levels of susceptibility or resistance of the potato cultivars and the prevailing weather conditions of the experimental locations. In this way, the negative effects inflicted to human being and environment due to misapplication and/or indiscriminate application of fungicides could be diminished and the burden of the economic costs for late blight control would be reduced.

6 Conclusions

- The Nicaraguan population of *P. infestans* is dominated by the clonal genotype NI-1, although four more genotypes have been detected at very low frequency. This clonal population of *P. infestans* is of the A2 mating type and the Ia mtDNA haplotype (**Paper I**).

- The Nicaraguan population of *P. infestans* is highly variable regarding race composition and has a high frequency of individuals resistant to metalaxyl. Unlike other countries in Latin America, this clonal lineage appears to dominate in both potato and tomato crops (**Paper I**).

- Although SSR fingerprinting and mtDNA haplotyping showed no differentiation between potato and tomato isolates, aggressiveness tests revealed that tomato isolates showed a general, but not exclusive, host-specificity and were more aggressive on tomato. In contrast, potato isolates showed host-preference toward tomato detached leaflets and were more aggressive on them (**Paper II**).

- The simulation model was considered adequate as it accurately predicted high disease severity in susceptible cultivars without fungicide protection, and demonstrated a decrease in the disease progress curves with additional fungicide applications, similar to that observed in the field plots. The model also generally predicted inadequate fungicide control, even with a 4-day spray interval, which also occurred in the field. Lack of adequate fungicide protection would indicate the need for cultivars with higher levels of durable resistance, and that farmers should consider more

effective fungicides applications (higher dosages or different chemistries) if susceptible cultivars are used (**Paper III**).

- Field and simulated experiments showed some trends about the relationship between host resistance and fungicide need. For any given final disease severity level, for potato cultivars with high susceptibility, the marginal effect of reducing susceptibility on the spray interval was small, while for potato cultivars with low susceptibility, the marginal effect was larger. In general, these trends could be used, although with cautions, to adjust the fungicide application intervals according to the resistance levels of the potato cultivars and the environmental conditions of the experimental sites (**Paper IV**).

7 Future perspectives

- Important issues such as survival of *P. infestans* from season to season, sources of primary inoculum that initiate epidemics at the beginning of the growing season and the role of the native solanaceous plants as a reservoir of inoculum remain unknown and could clarify the reasons behind the survival and persistence of the dominant clonal lineage despite probable incursions by other genotypes.

- In the near future, it is recommended to monitor more extensively and more systematically *P. infestans* populations from Nicaragua to detect possible population shifts due to the processes that govern pathogen evolution such as mutation, migration (gene and genotype flow), genetic drift, mating system (reproduction) and selection.

- The LB2004 simulation model should be modified to simulate the effects of other fungicides that pose a lower health and/or environmental risk, and are potentially more effective, including those with systemic or translaminar properties.

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