Population structure and pathogenicity evolution of *Phytophthora infestans* affects epidemiology and management of late blight disease

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2019

Acta Universitatis agriculturae Sueciae
2019:14

Cover: A potato crop heavily infected by *Phytophthora infestans* in a field in Kenya (photo: A. Njoroge)

ISSN 1652-6880
ISBN (print version) 978-91-7760-346-7
ISBN (electronic version) 978-91-7760-347-4

Print: SLU Service/Repro, Uppsala 2019

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Abstract

Sound management of late blight, the disease caused by the notorious oomycete pathogen *Phytophthora infestans* (Mont.) de Bary, is dependent on the pathogen's population biology. However, for *P. infestans* population structure to give guidance for disease management, successful information flow between the researchers and the practitioners is paramount. We analysed the population in eastern-Africa to determine the pathogen genotypes present in the region. We characterized the isolates using microsatellite markers and mitochondrial DNA haplotypes to enable comparisons with global populations. A European lineage, 2_A1 was found to be dominating the population in eastern-Africa. In addition, the 2_A1 lineage was found to be more aggressive in terms of lesion size, latent periods and incubation periods when compared to the old US-1 lineage. We thus concluded that the tested aggressiveness traits could have partly contributed to the quick displacement of US-1 by 2_A1 in the region. In a study predicting host durability of a genetically engineered potato with a stack of three resistance genes as well as a conventionally bred potato with a stack of five resistance genes, the assessment of pathogen effector genes proved valuable to deduce which of the *R*-genes were functional in the field. From the effector study, it can be concluded that effector genes in target local *P. infestans* populations should inform selection of breeding materials since globally, pathogen populations are very diverse. An assessment of commonly grown potato cultivars in eastern-Africa to quantify their susceptibility to late blight in the field found out that nearly all cultivars had partial resistance to *P. infestans.* The growers' choice of cultivars is to high degree governed by market demands. Unfortunately, many cultivars with good resistance to late blight have other undesirable agronomic traits hence the rationale behind growing cultivars that are highly susceptible to late blight. Disease management practices, host durability prediction tools and potato breeding approaches should be suitably adjusted to the existing pathogen population.

Keywords: late blight, SSR-genotyping, gene pyramiding, effectors, host resistance

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Dedication

To my family, for being the pillar in my life

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List of publications

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

I Njoroge, A.W., Andersson, B., Lees, A., Mutai, C., Forbes, G.A., Yuen, J.E. & Pelle, R. (2018). Genotyping of *Phytophthora infestans* in eastern-Africa reveals a dominating invasive European lineage. *Phytopathology*. doi.org/10.1094/PHYTO-O7-18-0234-R

II Njoroge, A.W., Andersson, B.,Yuen, J.E. & Forbes, G.A (2018). Greater aggressiveness in the 2_A1 lineage of *Phytophthora infestans* may partially explain its rapid displacement of the US-1 lineage in east Africa. *Plant Pathology*. doi: 10.1111/ppa.1297

III Njoroge, A.W., Ghislain, M., Andersson, B., Magembe, E., Mutai, C., Pelle, R., Yuen, J.E. & Forbes, G.A. (2018). Predicting durability of host resistance to late blight disease via effector screening in eastern-Africa *Phytophthora infestans* population. (Submitted).

IV Njoroge, A.W., Andersson, B., Yuen, J.E. & Forbes, G.A. (2018). Quantifying levels of late blight susceptibility in some potato cultivars found in east Africa. (Manuscript).

The contribution of Anne Njoroge to the papers included in this thesis was as follows:

I Planned the study with the co-authors. Collected all the field samples and did all the laboratory analyses. Analysed the data and wrote the manuscript with the help of the co-authors.

II Planned and performed the experimental work with the help of the co-authors. Analysed the data and wrote the manuscript with the help of the co-authors.

III Planned the study with the co-authors. Collected the field samples and did all the laboratory analyses. Analysed the data and wrote the manuscript with the help of the co-authors.

IV Planned the experiment with the co-authors. Carried out the field trials and collected the field data. Analysed the data and wrote the manuscript together with the co-authors.

1 Introduction

Late blight caused by the oomycete pathogen *Phytophthora infestans* (Mont.) de Bary is the most important disease on potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) globally since it causes very serious, direct and indirect, crop losses. The disease became infamous in the mid 1800's when the demography of Ireland was changed forever after one million people died and another million emigrated to North America after the destructive effects of a potato famine, for which late blight was the proximate cause (Bourke, 1993). Even to date, devastating late blight epidemics on tomato and potato are still a global occurrence (Fry, 2008). In eastern-Africa, the situation with severe late blight epidemics is the same despite endless efforts by scientists and other stakeholders to develop and implement various disease management options. Their struggles have often been rendered ineffectual due to the ability of *P. infestans* to rapidly overcome common control methods.

In this thesis, I propose that late blight management can be greatly enhanced if an understanding of the causal pathogen population is used in the development of the control strategy to be employed. Knowing the exact strains of *P. infestans* causing late blight in eastern-Africa, together with their genotypic and phenotypic traits, will enable stakeholders in making better-informed disease management choices that are suited for the local pathogen populations.

2 *Phytophthora infestans* and the late blight disease

2.1 History and origin

The origin of *P. infestans* is disputed to be either central Mexico or the Andean region in South America. Mexico is proposed to be the center of origin due to the occurrence of the two mating types, high genetic and phenotypic diversity as well as the presence of two close relatives, *Phytophthora mirabilis* and *Phytophthora ipomoeae*, in that region (Goss et al., 2014; Grünwald and Flier, 2005). However, Gómez-Alpizar et al. (2007) reports that *P. infestans* came from the Andes since that is the center of origin of cultivated potato. Studies there have revealed variations of Andean *P. infestans* lineages as well as the presence of *Phytophthora andina*, a close relative of *P. infestans*. In eastern-Africa, the disease was first found in Kenya by Dr. Nattrass in 1941. A year later, late blight had spread to the foothills of Mount Elgon on the Kenya-Uganda border from where it spread westward into Uganda and into the democratic republic of Congo and finally southward into Tanzania (Natrass, 1944).

2.2 Taxonomy and Biology

The organism, *P. infestans*, is a eukaryote in the kingdom Stramenopila in the phylum Oomycota. *Phytophthora* is the largest genus in the order Peronosporales and as an oomycete, *P. infestans* is not considered as a fungus since oomycetes have cell walls composed of cellulose and store their energy as starch (Rossman and Palm, 2006). The *Phytophthora* genera contain more than 140 species which are divided into ten phylogenetic clades comprising both saprophytic and plant pathogenic species (Yang et al., 2017). Of these clade 1 comprises 13 species including *P. infestans* (Kroon et al., 2012). Other species

in clade 1 that are said to be close relatives of *P. infestans* include *P. andina*, that has actually been shown to be a hybrid between *P. infestans* and an unknown clade 1c parent (Goss et al., 2011), as well as *P. ipomoeae* (Flier et al., 2002). As a clade 1c member, *P. infestans* has amphigynous antheridia and semipapillate zoosporangia, which develop on distinctly differentiated sporangiophores (Kroon et al., 2012).

The biology of *P. infestans* has characteristics similar to other oomycetes that are diploid and coenocytic, lack chitin in the cell walls and have the ability to produce motile, biflagellate zoospores (Fry et al., 1993). *Phytophthora infestans* is heterothallic with two mating types, designated A1 and A2, and can undergo either sexual or asexual reproduction. The two sexual structures, antheridia (male organ) and oogonia (female organ), are induced if the two mating types coexist and infect the same plant tissue or are grown on the same artificial media. Fusion of the antheridia and the oogonia results in the formation of oospores, the sexual spore. The oospores possess a thick walled resistant cell which provides a means of long-term survival (Mayton et al., 2000). Since the oospores are formed via genetic fusion, they provide a means of genetic variability. They also act as initial source of inoculum to start an early infection since they are mainly soil-borne (Lehtinen and Hannukkala, 2004; Andersson et al., 1998; Evenhuis et al., 2007). The oospore germinates either directly by a germ tube with or without a sporangium on the end, or indirectly by the formation of a vesicle with zoospores. The ability to cause infections directly or indirectly is a trait that is temperature dependent with zoospores being produced at cooler temperatures (Fry and Grünwald, 2010). In the absence of both mating types, *P. infestans* can form specialized hyphae called sporangiophores, which emerge through the stomata of the stems and the leaves to asexually form sporangia. Zoosporangia can be formed in the presence of leaf wetness which is common in the morning when it's getting warmer and there is a drop in humidity. Due to the high number of the asexual sporangia which play the major role in spreading *P. infestans*, dispersal by wind or rain to nearby plants or neighbouring fields is very rapid (Fry and Goodwin, 1997). In fact, the disease has been referred to as a community disease due to its ability to spread rapidly from one field to another. The ability of *P. infestans* to be airborne plays an important role in late blight epidemiology as inoculum can easily get deposited in neighbouring fields to start an epidemic. However, long distance dispersal of the pathogen is hindered by the inability of the sporangia to survive the effects of solar radiation and low air humidity (Mizubuti et al. 2000). When zoospores or sporangia from infected foliage come into contact with tubers (Lacey, 1965; Lacey, 1967), infection of the tubers through buds, lenticels or wounds results (Jones et al.,

1912; Zan, 1962). Blighted potato tubers provide a mechanism for survival between cropping seasons as well as long distant spread of the pathogen.

Phytophthora infestans has also been classified according to physiological races depending on the ability to attack different cultivars of the same host species, each presumably containing single resistance (*R*) genes. As such, strains have been classified into races depending on their ability to infect known *Solanum demissum* resistance genes (Jones et al., 1912). Race 0 strains cannot attack cultivars with any of the resistance genes. Races able to attack one or two *R*-genes are regarded as simple while those with a wider virulence spectra are complex (Leonards-Schippers et al., 1992). However, there are suggestions to revise the race nomenclature since it only applies to potato cultivars comprising the *S. demissum R*-genes and many other wild *Solanums* have been used to obtain other resistance genes (Vleeshouwers et al., 2011; Pankin et al., 2012).

2.3 The hosts

In eastern-Africa, late blight is continually reported on potato and tomato but in 1950, *P. infestans* was reported to appear on the leaves of perennial woody *Solanums* (*S. indicum, S. panduraeforme* and *S. incanum*) and a tree-like *S. aculeastrum* (Nattrass and Ryan, 1951). The importance of the wild *Solanums* is that they thrive in the wetter forest areas where late blight conditions are always favourable and hence, they could act as sources of lasting inoculum. In other regions of Africa, other *Solanums* like *Petunia x hybrida* (Hort) and garden huckleberry (*Solanum scabrum* Mill.) have been reported to be infected by *P. infestans* (Pule et al., 2013; Fontem et al., 2005). The presence of late blight on *Petunia x hybrida* is of economic importance under greenhouse conditions (Deahl et al., 2003; McLeod and Coertze, 2006). On garden huckleberry, a popular traditional vegetable crop in west and central Africa, *P. infestans* isolates on this host readily infect potato and tomato indicating the occurrence of epidemics from cross-infections (Fontem et al., 2005).

Potato and tomato can be found growing all year round in eastern-Africa due to suitable tropical conditions. The presence of the two hosts all the time makes it easy for *P. infestans* to survive between seasons. As a consequence, wherever potato or tomato plants are found, some late blight attacks can always be observed, except during extremely dry seasons. When weather becomes favourable, pathogen attack can happen at any stage of plant development.

2.3.1 Potato

Potato originated in the Andes of south America (Spooner et al., 2005). The British farmers and colonial officials introduced the crop to Kenya and other areas of eastern-Africa during the 1880's (Hijmans, 2001). Late blight on this host was first reported on a cultivar Kerr's Pink whose seed had been imported from the United Kingdom for the 1941 cropping season in Kenya (Nattrass, 1944). The disease completely destroyed two potato cultivars that had been grown in the region for a long time and also caused devastating effects to the European cultivar Kerr's Pink. The importation and testing of late blight resistant potato cultivars began in 1943 (Wallace and Wallace, 1945) and has continued to date. The potato cultivars tested at the time were resistant to attack by *P. infestans* races 0 and 2,4 which were present at the time but eventually new races 4; 1,2; 2; and 1,3,4 were reported to attack the potato crop (Wallace and Wallace 1945). On potato, the pathogen mostly affects the foliage, but it can also affect stolons and tubers (Figure 2). Although tuber blight is not common, instances of late blight infected tubers even among ware potato have been found (Figure 2d). Tubers get infected during handling and in-store spread of infection to healthy tubers is common (Dowley and O'Sullivan, 1991). In fact, tubers are a means of long distance dispersal of *P. infestans* (Abad and Abad, 1997; Nyankanga et al., 2004).

Figure 1: Typical late blight symptoms during the early stages of an epidemic (1a); irregular necrotic lesions originating from the leaf-stalks (1b); necrotic lesions on the apical stems (1c) and brown-rusty symptoms caused by *P. infestans* on a tuber found amongst ware potato traded in an open air market in Kenya (1d). Photos A. Njoroge.

2.3.2 Tomato

Tomato, (*Solanum lycopersicum* L.) (formerly known as *Lycopersicon esculentum* Mill), is a major vegetable crop worldwide (FAOSTAT, 2011). Tomato origins can be traced back to the Andes of South America (Jenkins, 1948) but there are two competing hypotheses on its domestication, one in Peru and another from Mexico (Peralta et al., 2006). It was introduced to Europe in the sixteenth century and is thought to have spread to other parts of the world from there (Razdan, 2006). In eastern-Africa, late blight on tomato was first reported in mid-1940's and of the blight races reported then, race 3 and 4 were identified on tomato (Wallace and Wallace, 1945). Not much on tomato late blight has been published for the region but it was reported that the common cultivars grown are highly susceptible to *P. infestans* (Tumwine et al., 2002). A host-specialized strain of the US-1 lineage is found on tomatoes in eastern-Africa (Njoroge et al., 2016; Vega-Sanchez et al., 2000). On tomato plants, *P. infestans* typically attacks the entire plant including the tomato fruits (Figure 3).

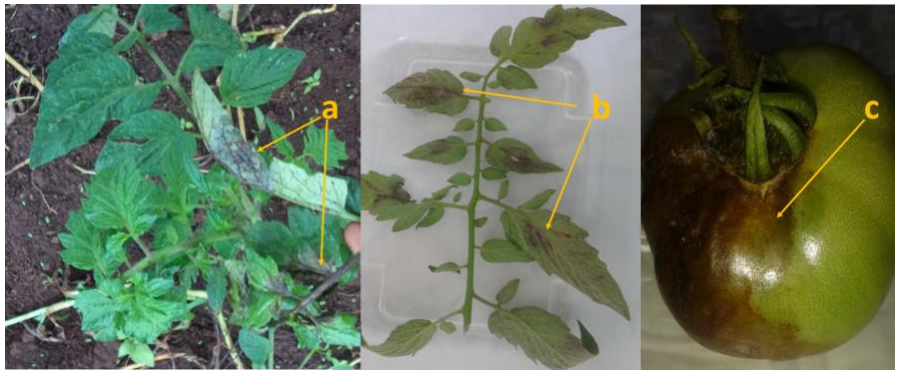

Figure 2. Typical late blight symptoms on the leaves of tomato plants (2a, 2b) and on a tomato fruit (2c). Photos A. Njoroge.

2.4 Global populations of *Phytophthora infestans*

Migrations of *P. infestans* from its center of origin to other parts of the world, probably starting in the 1970's, have resulted in changes in global population structure of the pathogen (Goodwin, 1997). In all parts of the world except Mexico, which is considered the center of origin, the population was dominated by a single A1 mating type lineage that was designated as US-1 (Goodwin and Fry, 1994). A migration event brought new genotypes to Europe (Hohl and Iselin, 1984), supposedly in a shipment of potato tubers from Mexico to Europe in 1976 (Niederhauser, 1991), which rapidly displaced the earlier genotypes (Drenth et al., 1994; Fry and Goodwin, 1997; Spielman et al., 1991; Sujkowski

et al., 1994). The tell-tale signs of *P. infestans* population shifts at the time was the difficulties encountered while managing potato late blight. In addition to the presence of both mating types, isolates resistant to Metalaxyl, a systemic fungicide that had provided excellent protection against strains of the US-1 lineage, arose during the 1980s (Davidse and et al., 1981). Analyses of herbarium samples collected between 1845 and 1896 indicated that before the dominance of the US-1 lineage, the *P. infestans* populations in Europe were dominated by a single genotype named HERB-1 (Yoshida et al., 2014). The HERB-1 lineage persisted for about 50 years before it was displaced by the US-1 clonal lineage (Yoshida et al., 2014). The US-1 lineage eventually spread panglobally from Europe (Goodwin, 1997). It is thus apparent that the *P. infestans* populations in Europe have undergone at least two migration events. The first happened in the mid-19th century introducing isolates, which did not include the A2 mating type, from the United States into Europe. The second event in 1976 introduced isolates of both mating types from Mexico into Europe.

2.5 *P. infestans* populations in sub-Saharan Africa

Through potato seed trade, new genotypes were distributed widely in Europe, to South America, North Africa and Asia (Fry et al., 2009). However, the new genotypes were not introduced into sub-Saharan Africa (SSA) at that time. Initial information from South Africa prior to 1980s show that only the A1 mating type was present (Smoot et al., 1958). Other A1 mating type genotypes other than the panglobally distributed US-1 lineage, were reported in mid-1990's in Rwanda (Forbes et al., 1998), in early 2000 in Ethiopia (Schiessendoppler and Molnar, 2002) and in 2007-2009 in Kenya (Pule et al., 2013; Were et al., 2013). Dominance of a new A1 mating type lineage in Kenya, designated by Pule et al. (2013) as KE-1 and by Were et al. (2013) as the 2_A1 lineage, was reported for the first time after the lineage managed to completely displace the US-1 lineage on potato in Kenya and eastern Uganda (Njoroge et al., 2016). The KE-1 and the European 2_A1 were confirmed to be the same lineage by Njoroge et al. (2016).

The variation in the *P. infestans* populations even in SSA is largely caused by seed tuber movement. In north Africa, both A1 and A2 mating types originating from imported seed from Europe, have been reported (Baka, 1997; El-Korany, 1994; Hammi et al., 2001; Sedegui et al., 2000). Though the other regions of Africa have been largely spared from the introductions of the new A2 mating type isolates, many countries are importing seed tubers from Europe and new pathogen genotypes via this route is expected to continue. Seed trade is the route believed to have brought the 2_A1 lineage into eastern-Africa, and there are reports of the presence of the A2 mating type (the 33_A2 genotype,

commonly found in Europe) in Nigeria (David Cooke, *personal communication*). Occurrence of the two mating types in SSA, a region that struggles to manage late blight might result to unbearable severe epidemics should *P. infestans* reproduce sexually.

2.6 Management of the late blight disease

Management strategies for late blight include two main approaches; the application of fungicides and host resistance. Additionally, use of disease-free tubers for planting and cultural control methods can greatly reduce the early onset of disease symptoms.

2.6.1 The use of fungicides

To the small-scale farmers in eastern-Africa, the use of fungicides to combat late blight seems like an acceptable norm and over-dependency on this practice threatens to compromise both the environment and human health. Most growers apply fungicides on the crops without personal protective equipment, causing them to over-expose themselves to the chemicals (Figure 4a). Large amounts of fungicide residues due to frequent applications are always evident on tomato and potato plants since growers who plant these crops for commercial purposes leave nothing to chance (Figure 4b). Despite the frequent fungicide use, late blight epidemics are increasingly more difficulty to manage due to occurrence of isolates resistant to modern fungicides hence the emphasis on the need for host resistance (Deahl et al., 1993; Goodwin et al., 1996; Grünwald et al., 2001). A newer fungicide in the region, Infinito (Fluopicolide and Propamocarb) is reportedly not able to manage late blight in the field by growers in Uganda (Gerald Baguma, *personal communication*) as well as in Kenya (Daniel Mbiri, *personal communication*).

In eastern-Africa, isolates of the US-1 lineage exhibiting high Metalaxyl resistance have been reported (Mukalazi et al., 2001). The spectrum for fungicide response of the 2_A1 genotypes is yet to be determined but considering its rapid spread in a region that heavily relies on chemical control, indications are that isolates insensitive to the commonly used fungicides exists. The European 33_A2 lineage now in west Africa is associated with reduced sensitivity to fluazinam, a non-systemic protectant fungicide but mancozeb, another commonly used protectant fungicide, is still effective for its management (Serge and Daniele, 2015). Depending on what fungicides are commonly used, the appearance of 33_A2 genotypes in west Africa could negatively affect the possibilities to manage late blight in SSA. These coupled

with other challenges associated with chemical control in the region such as high cost of fungicides, fungicide adulteration, the use of low dosages and expired products, could result in severe epidemics caused by 33_A2.

Figure 3. A grower without personal protective equipment spraying potato plants (3a). A small tomato field heavily sprayed with fungicides, the sachets seen on the sticks were the ones containing the fungicides (3b). Photos A. Njoroge.

2.6.2 The use of host resistance

Host resistance is an observed phenotype in which a pathogen is less able to cause disease on the host. Development of durable resistant cultivars would thus be key for a sustainable late blight control measure (McDonald and Linde, 2002). Importantly, the societal outcry to minimize chemical use to manage diseases makes the use of host resistance a priority. Two types of host resistance to late blight have been described in potato, horizontal resistance and vertical resistance.

Horizontal or general resistance is said to be polygenic and slows the development of the pathogen (Leonards-Schippers et al., 1994; Peralta et al., 2006; Umaerus and Umaerus, 1994). Since this resistance is strongly correlated with maturity type (Bormann et al., 2004; Simko, 2002) it creates problems for late blight resistance breeding (Wastie, 1991).

Vertical or specific resistance confers immunity or near immunity to the plant through a hypersensitive response and is said to be monogenic. The genes conferring this resistance are called *R*-genes and are thought to produce proteins involved in pathogen recognition and the initiation of defense responses. In the early 1900's, breeders introgressed *R*-genes from the Mexican wild species *Solanum demissum* Lindl. into cultivated potato with great success (Müller and Black, 1952). Unfortunately, these genes were readily overcome by *P. infestans* races when deployed in potato cultivars making the resistance they confer to

have poor durability (Wastie, 1991). Although these *R*-genes have contributed little to practical late blight management, reports indicate that such *R*-genes might have a beneficial effect as resistance in clones with field resistance increases if they also have *R*-genes (Stewart et al., 2003). Moreover, stacking *R*-genes in a single crop variety has been shown to increase disease resistance (Haesaert et al., 2015; Haverkort et al., 2016).

As races of *P. infestans* overcame the resistance obtained from *S. demissum*, researchers turned to other *Solanum* species for resistance genes. Such alternative *R*-genes includes *Rpi-ber* from *Solanum berthaultii* (Ewing et al., 2000; Rauscher et al., 2006), *Rpi-moc1* identified in *Solanum mochiquense* Ochoa (Smilde et al., 2005), *Rpi-phu1* from *Solanum phureja* Juz. et Buk. (Śliwka et al., 2006) and *Rpi1* from *Solanum pinnatisectum* Dun. (Kuhl et al. 2001). *Solanum bulbocastum* has yielded several *R*-genes, *Rpi-blb1* / *RB* (Song et al., 2003; van der Vossen et al., 2003), *Rpi-blb2* (van der Vossen et al., 2005) and *Rpi-blb3* (Park et al., 2005). Additionally, *Rpi-vnt1.1*, *Rpi-vnt1.2* and *Rpi-vnt1.3* were identified from *Solanum venturi* (Foster et al., 2009).

2.7 Engineering host resistance

Incorporation of resistance to diseases during the development of crop cultivars is one of the challenges breeders have to deal with. Conventional breeding methods utilizing crosses made between resistant and susceptible parents and thereafter evaluating the large progeny populations under disease conducive conditions has been the path to incorporating disease resistance genes into plants. Currently, genetic modification (GM) techniques has allowed the introduction of genetic material into existing potato cultivars to the absolute minimum required to achieve the desired trait (Haesaert et al., 2015). Cisgenes, the natural indigenous potato genes or those from crossable species, can be used in breeding programs to make natural crosses with potato (Haverkort et al., 2016). Cisgenic plants are highly similar to natural potato, especially if foreign genetic material such as selectable marker is absent, and the only difference is the way by which the genes are introduced (Haesaert et al., 2015). A genetic engineering approach also allows the efficient transfer of multiple *R*-genes. Stacking of several cisgenes is expected to confer durable resistance to late blight and this strategy should avert what happened in the past when resistance of single genes was broken rapidly. However, to be able to predict and monitor the durability of the cisgenes, prior information on the prevailing avirulence (*Avr*) genes in the pathogen population is paramount. This is because *R*-genes must be chosen such that they recognize different avirulence genes in the pathogen so as to have a wider resistance spectrum.

Genome editing using the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9(CRISPR-associated protein-9 nuclease) would be more preferable as it based on RNA-guided engineered nucleases rather than the introduction of foreign genes. The transfer of transgenes usually involves the use of selectable markers to monitor the transfer success rates. Since the markers are mostly antibiotics, this raises a lot of societal concerns as there is fear that these will affect the human health. Since resistance to *P. infestans* is recognition-dependent where the pathogen must be recognized by the host *R*-genes, genome editing of *R*-genes might be difficult. In that case therefore, the CRISPR-cas9 technology can only be used to edit the susceptibility genes in the host as altering the resistance genes could result to a gain of virulence in the pathogen. However, it is not known if changing the susceptibility genes will result to complete resistance phenotypes as seen from gene stacking. Attempts to develop a CRISPR/Cas for editing the *P. infestans* genome have not been successful yet (van den Hoogen and Govers, 2018).

2.8 Recognition dependent disease resistance

Plants have the capacity to resist potential attack via a highly effective defence system that involves recognition of the pathogens through strategies involving both conserved and variable pathogen elicitors (Dodds and Rathjen, 2010). The first line of the plants' immune defence involves recognition of conserved elements of the invading microbe, designated as microbe-associated molecular patterns (MAMPs) which includes bacterial flagellin or fungal chitin, by the plants non-specific receptor proteins called pattern recognition receptors (PRRs) (Boller and Felix, 2009; Sharpee and Dean, 2016). Plants also respond to endogenous molecules such as cell wall or cuticular fragments, called danger-associated molecular patterns (DAMPs), that are released by the pathogen during invasion (Dodds and Rathjen, 2010). Molecular transmission between PRRs and MAMPs, or between PRRs and DAMPs, triggers a defence response called MAMP-triggered immunity (MTI), or DAMP-triggered immunity (DTI), which is able to prevent attacks by a wide range of pathogens. However, in the continuous coevolution between microbes and their associated hosts, pathogens acquired the ability to deliver secreted proteins, called effectors, that not only block MTI and DTI but also alter processes like host metabolism for the benefit of the invading pathogen (Bozkurt et al., 2012; Sharpee and Dean, 2016). As a counter measure to this, the plants have developed surveillance proteins, the products of their *R*-genes, to directly or indirectly monitor the presence of the pathogen effector proteins and avoid pathogen infection, a condition called effector-triggered immunity (ETI) (Jones and Dangl, 2006). ETI involves

detection of specific avirulence (AVR effectors) directly through direct ligand-receptor interactions or indirectly through detection of effector action on host targets (Dodds and Rathjen, 2010; Liu et al., 2013). The ETI response often results to a programmed cell death (PCD) (Jiang and Tyler, 2012) manifested by a hypersensitive response (HR) localized to infection sites to kill the invading pathogen (Chisholm et al., 2006; Jones and Dangl, 2006). MTI and DTI are generally effective against non-adapted pathogens and results to non-host resistance whereas ETI is only effective against adapted pathogens. The outcome of these interactions largely depend on the elicitor molecules present in each infection (Dodds and Rathjen, 2010).

2.9 Virulence activities of pathogen effectors

Pathogens manipulate the defence response in plants via secretion of virulent effector molecules resulting in effector-triggered susceptibility (ETS) (Jones and Dangl, 2006; Dodds and Rathjen, 2010). The effectors operate at two main locations: in the host cell, the cytoplasmic space, and in the apoplastic space between adjacent cells (Giraldo and Valent, 2013). So, two classes of effector proteins, apoplastic effectors, secreted into plants' extracellular space, and cytoplasmic effectors, translocated inside the plant cell, are used by the pathogen to target distinct sites in the host plant (Birch et al., 2006; Kamoun, 2006).

The cytoplasmic effectors alter plant metabolism, signaling pathways and gene transcription to manipulate the plant defence response (Sharpee and Dean, 2016). During the alteration to the plant metabolic pathway, the pathogen effectors reduce salicylic acid (SA) levels hence lack of expression of antimicrobial *PR* (*pathogenesis-related*) genes in the unaffected host tissue to protect the rest of the plant from infection (Fu and Dong, 2013). SA, an essential plant hormone that mediates MTI, is a key player for systemic acquired resistance (SAR) which prepares the plant for pathogen attack (Fu and Dong, 2013; Sharpee and Dean, 2016). Equally, effectors affect jasmonic acid (JA) signaling, another important plant hormone involved in plant defence responses, that contributes to ETI (Liu et al., 2016). Effectors have been shown to suppress RNA silencing thus enhancing susceptibility to *Phytophthora* infections (Qiao et al., 2013; Qiao et al., 2015). Filamentous pathogens have also been shown to use their effectors to directly affect transcription factors and protein kinases in order to down-regulate genes involved in defence responses (Sharpee and Dean, 2016; Dodds and Rathjen, 2010). Cytoplasmic effectors that trigger crinkling and necrosis of leaves, the so called crinklers or CRN proteins (Torto et al., 2003), affect the reactive oxygen species (ROs) whose role is to damage the pathogen hence playing an important function in MTI (O'Brien et al., 2012).

Effectors have the ability to physically block or alter the necessary components of defence from reaching their intended target (Sharpee and Dean, 2016).

The apoplastic effectors include enzyme inhibitors and small cysteine-rich proteins that contribute to counter-defense by inhibiting host enzymes that accumulate in response to pathogen infection (Tian et al., 2005; Tian et al., 2004; Rose et al., 2002). The pathogens use these effectors to protect the invading hyphae from plant-produced hydrolytic enzymes hence blocking the triggering of MTI (De Jonge et al., 2010). Some effectors achieve virulence by competing for chitin-elicitor binding PRR proteins that mediate MTI through the recognition of chitin, a MAMP, during pathogen invasion (Kaku et al., 2006; Miya et al., 2007; Lo Presti et al., 2015). Other effectors have the capacity to inhibit glucanase enzymes, produced by the plant, in order to block MTI and any other anti-microbial activity the enzymes might have (Rose et al., 2002; Sánchez-Rangel et al., 2012). The papain-like cysteine proteases (PLCP), secreted from the plant into the apoplast during infection, are activated by the presence of salicylic acid and are able to induce *PR*-gene expressions and trigger host cell death during pathogen attack (van der Hoorn and Jones, 2004). A large number of filamentous pathogen effectors inhibit the activity of numerous PLCPs. An example is the AVRblb2 effector of *P. infestans* which accumulates around haustoria during infection and interacts with cysteine protease *C14*, preventing its secretion into the apoplast and thus rendering plants susceptible to late blight (Bozkurt et al., 2011).

2.10 Pathogen effector evolution

The direct and indirect interaction mechanisms between AVR and R proteins as well as the virulence functions of AVR proteins affect *Avr* gene evolution in nature (van der Hoorn and Kamoun, 2008). In the co-evolutionary battle between plants and their associated pathogens, generally pathogens have an added advantage relative to their host due to their shorter generation time and large population sizes (Zhan et al., 2014). The co-evolution process is thought to occur in natural ecosystems where plant and pathogen exhibit gene-for gene interactions (McDonald, 2004). For pathogens to survive upon deployment of new *R*-genes, they must transform new effector genes (*Avr* gene mutation) governing their virulence. The virulent pathogen races in turn gets selected resulting in the breakdown of host resistance (Chattopadhyay and Singh, 2017). In intensified agricultural systems, there is genetic uniformity in the host populations which results to continuous selection for virulent pathogen races (Stukenbrock and McDonald, 2008). Some virulence evolution mechanisms include diversifying selection and polymorphism with high rates of non-

synonymous substitutions, which alter amino acid sequences of pathogen avirulence proteins and consequently loss of recognition in response to the deployed *R*-genes (Ravensdale et al., 2011; Giraldo and Valent, 2013).

The best-studied cytoplasmic effectors of the class RxLR-dEER gene family, named after a four amino acid (Arginine, any amino acid, Leucine, Arginine :RxLR) motif common among oomycete avirulence (AVR) proteins, are recognized inside the host cells (Rehmany et al., 2005). The RxLR-effectors have an N-terminal domain that consists of a signal peptide, an RxLR-like motif, an optional amino acid motif (consisting of two glutamic acid residues and an arginine residues, often preceded by an aspartic acid residue) known as the dEER-motif, and a carboxyl (C)-terminal effector domain (Stassen and Van den Ackerveken, 2011). The N-terminal motif is similar in sequence, position and function to the host-cell targeting signal (PEXEL/HT motif) required for translocation of proteins from animal parasitic plasmodia into red blood cells (Bhattacharjee et al., 2006; Birch et al., 2006). The crinkler effectors motif, also called the CRN motif, occurs more frequently in oomycetes (Schornack et al., 2010). The RXLR-dEER and CRN motifs function as signals for translocation into the host cytoplasm (Whisson et al., 2007; Oliva et al., 2010). The C-terminal region of the effectors is associated with the biochemical activity of the proteins inside plant cells (Schornack et al., 2009) and it is the main target for the adaptive evolution forces that drive the antagonistic interplay between pathogenic oomycetes and their host (Win et al., 2007).

Single nucleotide-polymorphisms (SNPs) within allelic forms of pathogen effectors, as is the case with *P. infestans* AVR3a effector, may give rise to proteins with changes in the amino acid which retain virulence function of the effector (Armstrong et al., 2005; Bos et al., 2010). Also, some effectors have achieved virulence by loss of a functional *Avr* gene, as reported for the truncated *Avr4* effector gene in *P. infestans* resulting from frame-shift mutations in the open reading frame (van Poppel et al., 2008). Some effector genes are maintained as diverse variants and lack of specific variants results in virulence on *R*-genes, as is the case with the *ipiO* gene of *P. infestans* (Champouret et al., 2009). Equally, presence of some allele variants, as reported for *Avrblb1* and *Avrblb2* in *P. infestans*, suggests they have evolved to avoid recognition by the cognate *Solanum R*-genes (van Poppel et al., 2008; Oh et al., 2009). Gene silencing has also been shown to be a mechanism of effector virulence evolution in *Phytophthora* plant pathogens (Foster et al., 2009; Vetukuri et al., 2013).

3 Population structure and pathogenicity evolution of *Phytophthora infestans* affects epidemiology and management of late blight disease

In eastern-Africa, late blight still devastates potato and tomato production systems since it was first reported in 1941. In Uganda, potato was practically wiped out by late blight in 1946 (Akimanzi, 1982). In Kenya, losses of about 40-80% have been reported depending on the cultivars and prevailing weather conditions (Lung'aho et al., 2008). Late blight was introduced into Rwanda and Burundi from Kenya and the disease is still very difficult to manage. Due to high disease pressure in the highland tropics, some farmers apply fungicides more than ten times per growing season (Namanda et al., 2004). The disease thus brings multiple costs plus the negative impact of pesticide use on human health and the environment.

The epidemiology and management of late blight disease is largely dependent on pathogen population structure and the host-pathogen interactions. This study has therefore monitored the pathogen population dynamics in eastern-Africa by examining isolates collected from diseased potato and tomato hosts over different seasons in different countries. The prevailing pathogen genotypes were identified genetically using microsatellite markers and mitochondrial DNA haplotypes while pathogen factors that may help the pathogen overcome resistance genes were screened with effector-specific primers. The fitness of the different genotypes was tested phenotypically by host inoculation studies under laboratory conditions and the evaluation of host resistance levels were assessed under natural infection pressure in field trials. The results will help to re-evaluate disease management measures by incorporating pathogen genetic and phenotypic traits for a better pathogen-informed control strategy. It is now possible to generate pathogen population data with the current advances in molecular biology which allow tracking migrations and changes in pathogen

composition using molecular typing tools. The insights from this research will help to improve food security in the region as a result of better management of late blight. Moreover, the information will be used to build a regional database for future disease surveillance.

3.1 Statement of the problem

Although varietal resistance to late blight does exist, farmers in eastern-Africa still grow potato cultivars that have low to moderate levels of resistance because these cultivars are highly valued by consumers (Nyankanga et al., 2004). For example, Victoria is a preferred cultivar grown in Uganda and Rwanda due to its short maturity period, but it has succumbed to *P. infestans* pathotypes over the years (Mukalazi et al., 2001). Even though other cultivars with some level of field resistance still exist, they are not commonly grown by farmers. In locations where disease pressure is high, a susceptible potato cultivar may require fungicide applications every 3–5 days. These foliar applications of fungicides result in very high input for pesticides in the potato and tomato production. Moreover, low affordability of fungicides for smallholder farmers and sub-optimal application practices results in frequent crop losses. Several resistant potato cultivars have been developed over the years but the vast majority of them are short-lived since the pathogen has a high potential to evolve new virulence genes (Erwin and Ribeiro, 1996) as late blight is a multi-cyclic disease with *P. infestans* completing multiple life cycles in a season. The biggest challenge of managing late blight therefore is the ability of *P. infestans* to undergo major population shifts in agricultural systems via the successive emergence and migration of asexual lineages (Cooke et al. 2012). Despite the considerable attention to introduction of potato clones and their evaluation for resistance, durable host resistance has been difficult to develop via conventional methods. Pyramiding of *R*-genes and their careful deployment over time is a promising strategy for reducing the devastating outcomes of late blight (Jo, 2013). The use of cultivars with several *R*-genes stacked together will minimize chances of *P. infestans* easily overcoming host resistance governed by single *R*-genes and this could be an approach to more durable host resistance to late blight. All these coupled with continuous assessment of the prevailing pathogen population in the eastern-Africa region, to better understand the genotypic and phenotypic traits of the *P. infestans* strains present in the region, will aid in re-designing late blight management strategies that are workable for the region.

4 Aims and scope of thesis

This study was designed to assess the pathogen population shifts in eastern-Africa and screen for pathogen effector genes that may help *P. infestans* overcome deployed host resistance. An understanding of pathogen population dynamics is sub-Saharan Africa will aid in designing disease management strategies that are suited for *P. infestans* populations in the region thereby effectively reducing losses due to late blight. Host resistance durability, for example, is wholly dependent on the dynamics of virulence in the local strains of *P. infestans.* Equally, some *P. infestans* strains in certain areas are insensitive to certain fungicide active ingredients. As such, there may be no resistance genes or fungicides that are globally effective. Reports of a new pathogen lineage of *P. infestans* in Kenya catalysed research on the late blight pathogen in the wider eastern-Africa region represented here by five countries namely, Kenya, Uganda, Tanzania, Rwanda and Burundi. The research commenced by quantifying the existing host resistance to *P. infestans* in some common potato cultivars grown in eastern-Africa (**Paper IV**). The objective was to use the late blight resistance ratings from different potato cultivars to assess how the shifting pathogen population would affect the existing host resistance. Pathogen population studies are rare for the region and the existing ones are mainly for individual countries or specific areas within a country. We assessed the population structure of the *P. infestans* using neutral markers to map what lineages were causing late blight in the different countries (**Paper I**). New *P. infestans* lineages are credited with increased levels of pathogenicity hence the need to investigate how far the new lineage had spread for better disease management designs. Certain phenotypic traits confer competitive advantages of new *P. infestans* lineages over the endemic ones in many regions. To try and understand why a new lineage had succeeded in competing and establishing itself in the region, we tested some aggressiveness traits which we thought might partly contribute to its fitness (**Paper II**).

Due to continuous breakdown of host resistance based on single *R*-genes, stacking several *R*-genes from wild potato relatives is an improved breeding strategy. Recognition dependent disease resistant is becoming increasingly important in the breeding for late blight resistant potato but information on effectors of targeted pathogen population is essential to monitor emergence of virulent pathogen races. The international potato center (CIP) has engineered a late blight transgenic potato for Africa with a stack of three *R*-genes obtained from wild potato relatives. The assumption that these genes will recognize the *P. infestans* isolates needed validation by screening the eastern-Africa *P. infestans* population for the presence and absence of the corresponding effector genes. Our study tested the effectors matching the *R*-genes in the CIP material, and, in addition, some *R*-genes present in a conventionally bred potato with a stack of five resistance genes, cv. Sarpo Mira, to determine if the *R*-genes were functional for eastern-Africa (**Paper III**). The functionality of the *R*-genes in cv. Sarpo Mira was predicted depending on whether the matching pathogen effector genes displayed sequence polymorphisms.

5 Methodology

The study involved sampling of diseased leaflets of potato and tomato leaflets in the major potato growing areas of five eastern-Africa countries (Kenya, Uganda, Tanzania, Rwanda and Burundi). In all the countries, the survey involved collections of single leaf lesions on FTA cards (Figure 4) obtained by crushing pieces of leaflets cut from the margins of actively spreading foliar blight lesions onto the cards and air-drying them before storage at room temperature. The FTA card samples were used for microsatellite genotyping as well as mitochondrial DNA haplotyping (**Paper I**). In Kenya and Uganda, additional sampling of infected potato leaflets with single lesions was carried out to isolate live samples of *P. infestans* for phenotypic assessment (**Paper II**). All *P. infestans* isolates in culture were studied in their country of origin (**Paper II**). Additionally, samples were collected in RNAlater solution from Uganda, Kenya, Rwanda and Burundi and used for effector gene expressions studies (**Paper III**). Field evaluations for cultivar susceptibility to late blight were only done in southwestern Uganda. The highlands of southwestern Uganda provide favourable weather for disease to thrive and the potato cultivars present in Uganda are also found in majority of the countries in eastern-Africa (**Paper IV**).

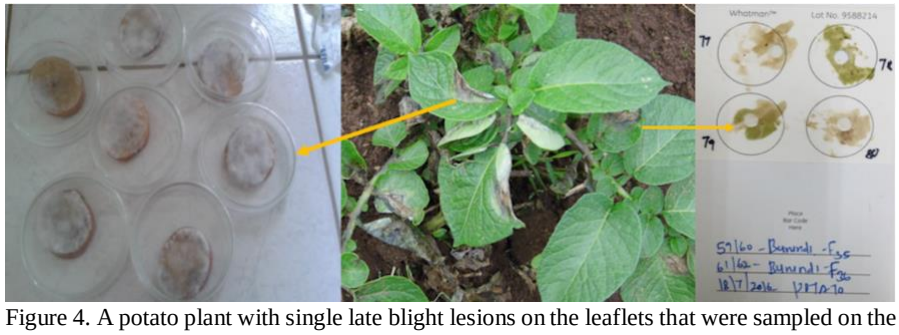

Figure 4. A potato plant with single late blight lesions on the leaflets that were sampled on the FTA card and put under tubers slices for *P. infestans* isolations. Photos A. Njoroge.

Late blight infected tomato plants were only sampled if they were found growing within the potato growing areas. Volunteer tomato plants with no fungicides applied on them were mainly targeted (Figure 5).

Figure 5. Volunteer tomato plants heavily devastated by late blight. Photo A. Njoroge.

6 Results and discussions

6.1 Genotyping of *Phytophthora infestans* in eastern-Africa reveals a dominating invasive European lineage (**Paper I**)

In the study reported here, we genotyped 1093 potato and 165 tomato samples from five eastern-Africa countries (Kenya, Uganda, Rwanda, Burundi and Tanzania) between 2013 and 2016. The results revealed the dominance of a European lineage, named 2_A1. This lineage is believed to have been introduced into Kenya from Europe via import of potato seed tubers although the exact time of introduction is unknown. However, estimates of its arrival are around late 2000's since it was first detected in 2007 in only two fields in Kenya (Pule et al., 2013). Additionally, two years after it was first detected, the 2_A1 lineage was found in more fields than the US-1 lineage in a subsequent study conducted in 2009 (Were et al., 2013). Our current study indicates that the 2_A1 lineage is not as diverse as the earlier dominating US-1 lineage. This supports the assumption that US-1 lineage has been present in the region for a long period of time, maybe even since the first late blight occurrences (**Paper I**). The US-1 lineage exhibited 85% multilocus genotypes (MLGs) diversity compared to 36% in the 2_A1 lineage. This indicates an accumulation of mutations which other than exhibiting large genotype diversity of the lineage, may have resulted to a progressive decline in the fitness of the US-1 as explained by Muller's Ratchet effect (Goodwin, 1997), which could partially explain the rapid takeover by a fitter 2_A1.

Overall, the genetic diversity of the *P. infestans* population in eastern-Africa is high (Table 1). Of the 773 samples of the 2_A1 lineage, 278 unique MLGs were obtained and only three of these occurred in three consecutive years, 2014-2016. Also, of these 278MLGs, seven similar MLGs occurred in three countries

with two of them appearing in Uganda, two in Kenya and three in Burundi. For a lineage that is new in a region, lesser diversity was expected and possibly a high number of MLGs shared amongst the countries. However, even at the time of the first discovery of the 2_A1 lineage in Kenya, relatively high diversity was evident (Pule et al., 2013). Also, of the 204 MLGs obtained from the 240 US-1 samples, only two of them occurred in subsequent years. One of the MLG occurred in 2014/2015 and the other in 2015/2016 indicating the higher variability of the US-1 genotypes in any subsequent years. However, no US-1 MLGs were shared amongst the five countries. This is probably expected of an old US-1 lineage which has co-evolved over the years and has been shaped by genotype by environment (GxE) interactions in the individual countries.

Table 1. The multilocus genotype diversity found in the *Phytophthora infestans* subpopulations on potato and tomato from samples collected between 2014-2016.

Population	N[a]	eMLG[b]	Hexp[c]
2_A1 potato	763	22.2	0.498
2_A1 tomato	31	27.0	0.508
US-1 Potato	161	29.9	0.527
US-1 tomato	80	28.9	0.497

[a] number of samples representing each subpopulation;

[b] expected number of MLGs for each subpopulation at largest shared sample size;

[c] Nei's gene diversity showing average genetic diversity per subpopulation.

Our study has also indicated a possible change in host-specialization of *P. infestans* lineage in eastern-Africa. Studies conducted in the region, including the current one, have showed that US-1 has genotypes specialized on potato and tomato (Njoroge et al., 2016; Vega-Sanchez et al., 2000). In the study reported here, discriminant analysis of principal components (DAPC) showed that the US-1 genotypes on potato formed distinct clusters away from the tomato genotypes (Figure 5, **Paper 1**). None of the US-1 MLGs were shared between potato and tomato. We also found 2_A1 genotypes for the first time on tomato in Kenya. The 2_A1 genotypes on potato and tomato clustered together indicating genetic similarity (Figure 5, **Paper 1)**. Seven MLGs of the 2_A1 lineage were found on potato and tomato. The similarity of the tomato and potato 2_A1 genotypes was found not only in Kenya since two of the seven MLGs appeared on potato in Uganda and three in Burundi. It is yet to be determined if infecting tomato with 2_A1 genotypes originating from potato causes less abundant sporulation and induces dark pigmentation on the potato leaves, a characteristic that has been reported to be stable and sufficient to differentiate

isolates belonging to potato or tomato populations of *P. infestans* (Vega-Sanchez et al., 2000).

The US-1 isolates from Tanzania had unique genotypes only present in that country. This was due to the presence of private alleles in one of the microsatellite markers. While this might indicate that the US-1 population in Tanzania could have been introduced from a different source, the history of late blight introduction in eastern-Africa (Cox and Large, 1960) seems to negate this line of thoughts. Moreover, the US-1 samples from potato and tomato from Tanzania clustered separately despite sharing the same private alleles which indicates existence of host specialization of the *P. infestans* in this country (Figure 2, **Paper I**). While similar potato cultivars are found growing in eastern-Africa, Tanzania seems to have a few other unique potato cultivars. Pathogen population structure can be influenced by its interactions with the host *R*-genes. We assume the genetic uniqueness of the Tanzania *P. infestans* population might be shaped by existing *R*-genes in that country. However, no reports linking variability of neutral markers to pathogen-host interactions exist hence we are not able to verify our claim. Nonetheless, the microclimate in Tanzania although unknown to us, might influence the genetic structure of *P. infestans* there.

Potato tuber movement is believed to be route that has enabled the 2_A1 lineage to establish in all the countries included in the present study. There is no formal seed tuber trading in the region but movement of ware potato to neighbouring countries is a frequent occurrence. For example, traders in southwestern Uganda will sell their freshly harvested potato tubers to Rwanda. Should any of these tubers carry infections of any potato pathogen, these biotic agents will be transported to the receiving country. Moreover, during conflicts, people move with farm produce across countries, which is another possible route for human-mediated pathogen movement. From our study, migration patterns were however unclear, since samples from countries that were farther apart, Kenya and Burundi, were more closely related than those from countries sharing land borders. Transfer of airborne inoculum between countries sharing land borders is however the likely route that has enabled the 2_A1 lineage to rapidly establish and dominate in all the studied countries.

When it comes to chemical control, most growers combine fungicides with the same mode of action, which can increase the risk of fungicide tolerance development in the *P. infestans* population. A high proportion of Metalaxyl resistant US-1 genotypes has also been reported in the region (Mukalazi et al., 2001). The presence of more aggressive strains of *P. infestans*, like the European 2_A lineage, in a region that employs suboptimal disease management practices, can result in late blight epidemics that are more difficult to manage.

6.2 Greater aggressiveness in the 2_A1 lineage of *Phytophthora infestans* may partially explain its rapid displacement of the US-1 lineage in east Africa (**Paper II**)

The displacement of the US-1 clonal lineage of *Phytophthora infestans* by the European 2_A1 lineage has been very rapid. Within a period of four years after the first discovery of 2_A1, complete displacement of the US-1 lineage on potato was evident in Kenya and eastern-Uganda (Njoroge et al., 2016). The ability of a pathogen genotype to displace other genotypes depends on its fitness, i.e, its ability to outcompete and contribute to the subsequent gene pool (Orr, 2009). Aggressiveness is one component of pathogen fitness and it refers to the quantitative components of the host-pathogen interactions (Andrivon et al., 1993). The US-1 population has presumably been present in easterns-Africa since the introduction of the disease in 1941 and it exhibits traits, like high Metalaxyl resistance, that would favour its competitiveness against other lineages (Mukalazi et al., 2001). Moreover, it has adapted to, and co-evolved with many different potato cultivars grown in eastern-Africa, most of which were released as resistant cultivars but eventually succumbed to late blight (Byarugaba et al., 2013; Olanya et al., 2001). This means US-1 has a wide virulence spectrum against the host resistance genes deployed in eastern-Africa. However, in many parts of the world, an increased problem of controlling late blight coincides with the displacement of the US-1 lineage by new more variable *P. infestans* populations (Spielman et al., 1991). This is because the new pathogen populations are marked by more aggressive genotypes of *P. infestans* (Day and Shattock, 1997). This is a parallel to the displacement of the US-1 lineage by the more aggressive 2_A1 lineage in eastern-Africa.

In this study, we quantified components of aggressiveness, namely: lesion size, latent and incubation periods for 2_A1 and US-1. The experiment was conducted in Kenya and Uganda on the detached leaflets of the potato cultivars Kachpot-1 and Sarpo Mira, and it revealed that 2_A1 genotypes were more aggressive than US-1 for all the aggressiveness components tested. For the leaf lesion sizes, the US-1 genotypes caused lesions that were 25% smaller than the 2_A1 genotypes. Equally for the incubation and latent periods, the 2_A1 genotypes produced late blight lesions and new sporangia in a shorter time compared to the US-1 genotypes.

We further tested the ability of the 2_A1 genotypes to infect tomato (Figure 6) since at the time of this study, no 2_A1 genotypes had been reported on tomato in the field. Host-specialization of the US-1 lineage on potato and tomato has been reported in eastern-Africa (Vega-Sanchez et al., 2000) as well as in other

parts of the world (Oyarzun et al., 1998; Ghimire et al., 2003). In eastern Uganda, the 2_A1 lineage had been found on potato while all the tomato isolates there were US-1 (Njoroge et al., 2016). This means that the 2_A1 lineage seemed not able to replace the host-adapted US-1 on tomato. Since all Kenyan isolates on potato were 2_A1, we used these isolates to infect tomato leaflets to assess to what extent this lineage would cause leaf lesions on this host (Figure 6). The results showed evidence of host preference since the potato 2_A1 isolates caused larger lesions on potato than on tomato. Whether the 2_A1 genotypes found on tomato in Kenya (**Paper I)** are host-specific is yet to be determined.

A tuber-slice assay was also included in this study to determine if the 2_A1 differed from the US-1 genotypes in their ability to cause tuber blight. New *P. infestans* genotypes have been reported to cause severe foliar and tuber blights when compared to the US-1 lineage. For example, the presence of the US-8 genotype in the USA and the 13_A2 genotype in Europe were characterized by increased aggressiveness on potato foliage and tubers (Cooke et al., 2011; Lambert and Currier, 1997). Tuber blight is said to act independently of foliar blight in potato cultivars even though it is also a factor associated with greater pathogenicity in *P. infestans* lineages (Oyarzún et al., 2011).

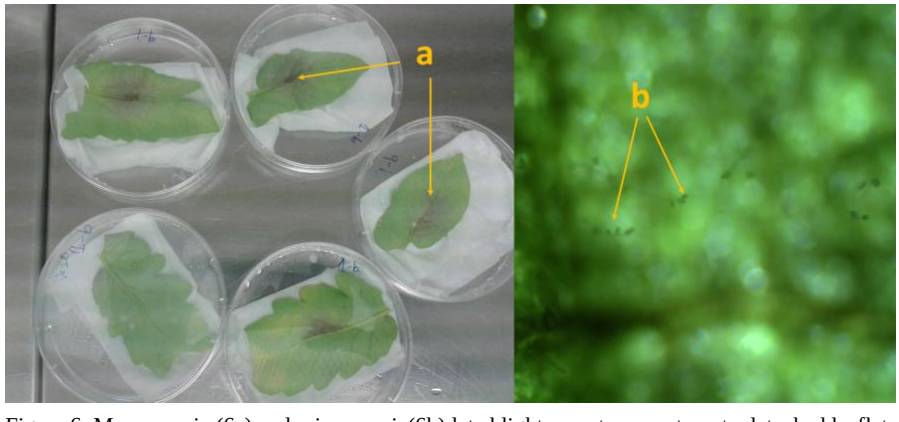

Figure 6. Macroscopic (6a) and microscopic(6b) late blight symptoms on tomato detached leaflets infected with *P. infestans* 2_A1 genotypes. Sporulating leaf lesions (6a) and sporangiophores with sporangia (6b). Photos A. Njoroge.

This study found out that though isolates within the US-1 and 2_A1 lineages varied significantly for tuber colony sizes, there were no differences between US-1 and 2_A1. The foliar and tuber assays were not correlated. Potato cultivars can vary greatly, and it might be important to screen a large number of potato cultivars grown in the region for tuber susceptibility to late blight. This is because tuber blight can have huge impact on potato production and latent

infection on tubers is a mechanism for long-term dispersal of new *P. infestans* genotypes (Abad and Abad, 1997; Nyankanga et al., 2004).

Continuous assessment of the 2_A1 lineage for pathogenicity traits as well as for fungicide insensitivity might provide information that can be used to better understand late blight epidemics in eastern-Africa. In Kenya, most growers are complaining of severe stem blight attacks even after fungicide application on their crop. The foliage other than the stems usually appear disease-free following fungicide treatments (Figure 7). The stem blight results to severe crop losses. Such incidences were unheard of when US-1 was the only lineage on potato. It thus seems the 2_A1 lineage has a means of fungicide avoidance and survival. The rise in stem blight will have direct impact on disease epidemiology. After harvesting, the vines are usually heaped on the sides of the farms and these cull piles become perfect places for *P. infestans* to survive between seasons. Moreover, the proximity of stems to the ground increases the risk of tuber infections.

Figure 7. A potato plant with a broken off stem due to late blight caused by *P. infestans*. The leaves and stalks look healthy due to fungicide treatments. Photo A. Njoroge.

6.3 Predicting durability of host resistance to late blight disease via effectors screening of eastern-Africa *Phytophthora infestans* population (Paper III)

An understanding of how *Phytophthora infestans* evades disease resistance is needed to advise the deployment of durable resistance. In this study, we examined the *P. infestans* population in eastern-Africa for presence / absence and variations of virulence factors (effector genes), that help the pathogen defeat deployed host resistance. Ever since the discovery of late blight, breeding for host resistance against *P. infestans* has been a never ending mission for potato breeders. In 1950's, optimism was high to find good host plant resistance when wild species in Mexico, especially *Solanum demissum*, were found to provide high levels of resistance or even immunity to *P. infestans* (Wastie, 1991). The resistance which was conferred by single genes (*R*-genes) was however qualitative, meaning it could only provide protection against specific pathogen races, and thus quickly eroded due to *P. infestans* evolution. Nevertheless, another type of resistance, which was deemed partial (quantitative or field resistance) was discovered (Bradshaw et al., 1995). While it is difficult to breed for partial resistance, a number of cultivars were released in eastern-Africa but many are not grown in large scale since they have some undesirable agronomic traits (Forbes, 2012).

Growers still prefer certain potato cultivars due to market demand hence the need to introduce different late blight *R*-genes into existing potato cultivars. The International Potato Center (CIP) has therefore pyramided three resistance genes via genetic engineering in farmer-preferred cultivars in eastern-Africa under the premise that *P. infestans* will not evade recognition by the three *R*-genes stacks. The 3*R* potato events remained late blight free under high disease pressure for four consecutive seasons in the field (Figure 8; Ghislain et al., 2018). We thus tested the 2_A1 and US-1 *P. infestans* isolates collected from Uganda, Kenya, Rwanda and Burundi for the presence and absence of the effector genes, *Avrblb1*, *Avrblb2* and *Avrvnt1* corresponding to the three *R*-genes, *Rpi-blb1(RB)*, *Rpi-blb2* and *Rpi-vnt1.1* in the 3*R* potato. The results showed the presence of avirulent effector transcripts, in both 2_A1 and US-1 lineages, that some of the *R*-genes in the 3*R* potato recognized to avert late blight development. Within the US-1 lineage, there were no effectors that would allow the functionality of the *RB* gene. The potato adapted US-1 had no *Avrblb1* effector gene whereas the tomato-adapted US-1 expressed the virulent *Avrblb1* (*IpiO4*) variant.

The results from this study confirms that pyramiding of *R*-genes can provide quantitative resistance against *P. infestans*. It was evident that only two of the *R*-genes in the 3*R* potato would work for a US-1 lineage *P. infestans* population. However, the fact that all resistance genes are present as a stack means that even though *RB* was not functional, the US-1 isolates could still not escape recognition by the *Rpi-blb2* and *Rpi-vnt1.1* genes. Had a preliminary study of the *P. infestans* population in eastern-Africa been carried out prior to the selection of the three resistance genes, it would have been noted that *RB* was not suited for the region. It is therefore important to study the biological function (including the effectors being recognized) of each *R*-gene individually before combining them in potato breeding transformation programs. Nevertheless, the uncertainty of *RB* functionality changed due to pathogen dynamics in eastern-Africa. The 2_A1 lineage which expresses all avirulent effectors that allow the functionality of the three resistance genes quickly replaced the US-1 lineage on potato. The 3*R* potato is thus currently effective against the dominating 2_A1 pathogen genotypes but the population should be continually monitored for occurrences of dynamic *P. infestans* races. In the Netherlands, an isolate able to overcome a stack of *RB* and *Rpi-blb2* has been reported (Förch et al., 2010).

Figure 8. A confined field trial with 3*R* transgenic potatoes being assayed for field resistance to *P. infestans* before late blight attack (8a) and one month after a severe late blight attack (8b). The brown patches (8b) are the non-transgenic control plants. Photos A. Njoroge.

While the tomato-adapted US-1 lineage still exists in all countries studied in eastern-Africa (**Paper I**), no known reports of infection of potato with a tomato-adapted US-1 in the field exists. We cannot however rule out a possible scenario of a host-jump in future where the tomato-adapted US-1 genotypes infect potato. However, if this ever happens, two of the 3*R* resistance genes would still be functional against the tomato-adapted US-1, since we found that the US-1 isolates from tomato had all the avirulent effectors matching *Rpi-blb2* and *Rpi-vnt1.1*. Nonetheless, the *RB* gene would be non-functional. While the 2_A1 lineage has moved to tomato in Kenya and isolates on the two hosts are genetically identical (**Paper I**), it will be important to determine if effector

composition varies within isolates collected from infected potato and tomato plants.

A European potato cultivar Sarpo Mira has been tested in the field in Kenya and Uganda (Figure 9). This cultivar has a stack of five resistance genes and it shows extreme resistance to late blight in the field (Rietman et al., 2012; Kim et al., 2012). In a detached leaf assay (DLA) however, genotypes of the 2_A1 and US-1 were able to infect cv. Sarpo Mira (**Paper II**). A similar DLA test in Sweden found that the genotypes there could not infect Sarpo Mira but rather hypersensitive responses were evident (Ali et al., 2012). These two scenarios are indicators of how different *P. infestans* populations in different regions can be.

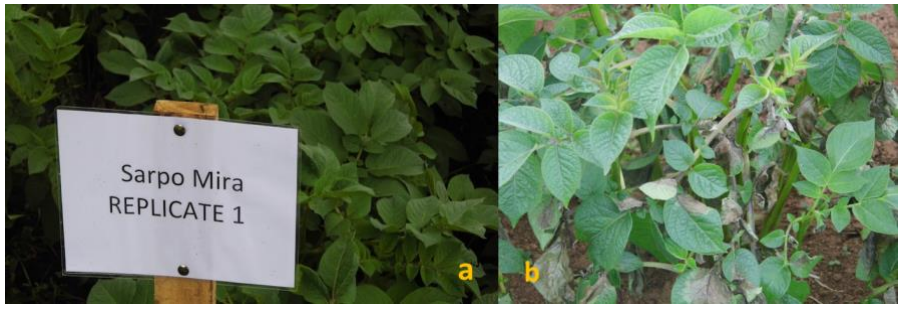

Figure 9. Potato cultivar Sarpo Mira (9a) without late blight infections versus a heavily infected potato plant (9b) in a field trial in Uganda. At maturity, cv. Sarpo Mira had no late blight symptoms while other cultivars had very severe late blight attacks. Photos A. Njoroge.

We tested for sequence variation of two effector transcripts, *Avr4* and *Avr8*, that correspond to and are recognized by two resistance genes, *R4* and *R8* / *Rpi-smira2* in cv. Sarpo Mira. The *Rpi-Smira2* which is a homolog of *R8* is credited for the field resistance in cv. Sarpo Mira (Rietman et al., 2012; Jo, 2013). Our results showed no variation in the *Avr8* effector transcripts after multiple sequence alignments against the reference *Avr8* transcript in the GenBank. For the *Avr4* transcripts, multiple sequence alignments revealed a frame shift mutation in the open reading frame in all the samples. This means while the *R8* would recognize *P. infestans* and prevent late blight development, the *R4* gene is non-functional since the mutated *Avr4* effector transcripts would synthesis truncated proteins that cannot be recognized by *R4* host gene. We therefore predict suitability of *R8* in host resistance breeding for eastern-Africa region. However, the durability of the resistance offered by *R8* will entirely depend on the biology of *P. infestans* in eastern-Africa since isolates that are able to escape recognition by *R8* have been reported (Rietman et al., 2012).

The assessment of effector genes should thus allow for detection of adaption within *P. infestans* populations for new virulence against newly introduced host

resistance. Most of the potato cultivars used in eastern-Africa have been bred elsewhere based on pathogen structures of those regions. Since pathogen populations are variable, suitability of the introgressed or engineered host resistance genes must thus be confirmed to work for targeted local *P. infestans* populations. Effector gene studies is therefore one way to predict suitability of new disease resistance genes even before they are deployed in the field.

6.4 Quantifying levels of late blight susceptibility in some potato cultivars found in east Africa (Paper IV)

Genetic resistance of potato cultivars to *Phytophthora infestans* is one of the many goals hoped for by potato breeding programs. In the past, potato cultivars with either specific or general resistance have been released in eastern-Africa but most have been abandoned by growers due to their high susceptibility to late blight (Forbes, 2012). *Phytophthora infestans* pathogen can completely overcome specific resistance which is governed by single resistance (*R*) genes (Flier et al., 1998 ; Flier et al., 2003). General resistance credited to the additive effects of many minor (*r*) genes, is said to be stable even though at times it does not result to a late blight free phenotype (Bradshaw et al., 1995).

Despite the availability of late blight resistant potato cultivars, growers still prefer the susceptible ones due to their market value (Forbes, 2012). Moreover, even the cultivars said to be resistant are only partially resistant and fungicides have to be used to avoid yield loss (Kromann et al., 2014). In this study we tested ten potato cultivars widely grown in southwestern Uganda namely, Victoria, Rwangume, Rwanshaki, Kimori, Kinigi, Rutuku, Bumbamagara, Kachpot-1, Cruza and Nakpot-5, for late blight susceptibility. The study was conducted for three consecutive seasons between 2013 and 2014. Rainfall patterns differed in the three seasons, making proper comparisons between seasons difficult. Our results do, however clearly indicate that cv. Victoria is the most susceptible and cv. Kinigi is the most resistant under local blight conditions (Figure 10). Overall though, all cultivars other than Victoria were moderately resistant as revealed by susceptibility score values of 1.7 to 4.3 calculated according to the method of (Yuen and Forbes, 2009). The method, based on an ascending-susceptibility analysis, estimates that zero disease represents the highest level of resistance.

We tried to estimate if the shift from the old US-1 to a dominating 2_A1 lineage in the pathogen population affected the potato cultivars ranking to late blight susceptibility. An ideal hypothetical scenario would be that the new *P. infestans* lineage, that is not adapted to the local conditions, would result in local cultivars having low susceptibility scores. This would be explained by the fact that the new pathogen genotypes have not encountered the existing host

resistance so initially the potato cultivars might display some level of disease resistance. After a while, the pathogen adapts and defeats the *R*-genes. We only compared results from season one and three since all isolates in the first season were US-1, while in the third season, 60% of the isolates were 2_A1. Although there was a reduction in susceptibility when the two seasons were compared, a stronger effect of pathogen genotypes on late blight scores should, theoretically, be evaluated when the entire *P. infestans* population is 2_A1. Such an assessment should be followed by other field evaluations to check if the 2_A1 fully defeats all the available host resistance. The indicator for such an outcome according to Yuen & Forbes (2009) would be very high susceptibility scores, on the resistance scale, for cultivars that now exhibit partial resistance.

Figure 10. A field trial to quantify *P. infestans* susceptibility of potato cultivars in east Africa. Potato plants early in the season (10a); cultivar Victoria (10b) and cultivar Kinigi (10c) towards the end of the season after a severe late blight attack. Photos A. Njoroge.

Despite the fact that breeding potato cultivars that combines necessary agronomic and market traits together with the quantitative resistance is difficult (Haverkort et al., 2009), some cultivars have been grown for many years without quantifying their late blight resistance (Yuen and Forbes, 2009). We can conclude from this study that despite using methods that allow for cross-locational and cross-seasonal assessments to explain evaluation procedures, unpredictable weather patterns play a big role in experimental outcomes. During the long rain seasons in eastern-Africa, the environment is highly conducive for late blight development to an extent that makes susceptibility of cultivars to be overestimated. Equally, the short rains can sometimes end abruptly, as was evident in season two in the present study, making the assessments incomplete.

Evaluating late blight progress helps to determine not only the differences amongst various potato cultivars but also finding dissimilarities in the same potato cultivar in separate cropping seasons. The findings from this research can be used not only in potato breeding but also in fungicide application programs.

7 Conclusions

The takeover of the European 2_A1 genotype in the *P. infestans* population in eastern-Africa in a changing wet and warmer climate, a favourable environment for *P. infestans* to thrive, could make late blight management more difficult. Despite using fungicides more frequently than before, growers are getting lower yields as cultivars that were previously released as resistant to late blight always ended up becoming susceptible.

- The eastern-Africa *P. infestans* population is highly variable despite the fact that it is composed of only two lineages, US-1 and 2_A1. The region is one of the few remaining areas to still report existence of the tomato-adapted US-1. However, in the recent past, eastern-Africa seems to be the first of the remaining areas with the tomato US-1 variant, to be experiencing displacement of the tomato US-1 by a lineage originating from potato (**Paper I**).
- Confirmation for greater aggressiveness of the 2_A1 compared to the US-1 lineage is evident. The conquest of the 2_A1 is probably due, at least in part, to the fact that it was more aggressive based on several parameters measured. Although there was genetic similarity observed between the 2_A1 genotypes on potato and tomato using microsatellite markers (**Paper I**), inoculation tests showed a preference to potato before tomato of the 2_A1 genotypes sampled from potato (**Paper II**).
- Effector gene studies predicted the stability of resistance of a transgenic potato, with a stack of three resistance genes, to the present *P. infestans* population in eastern-Africa, based on matching avirulent effectors to the introgressed host resistance genes. The suitability of the *R8* gene in late blight management was confirmed by the presence of nonpolymorphic *Avr8* effector transcripts in the local *P. infestans* genotypes. However, the *R4* gene is unsuitable for the region as evidenced by the presence of a frame shift mutation, in the open reading frame, in all the eastern-Africa isolates tested (**Paper III**).

- Field evaluations for late blight susceptibility showed that all the potato cultivars tested in eastern-Africa exhibited some partial resistance in the field when compared against the susceptible check. The incomplete displacement of the old US-1 lineage by the new aggressive 2_A1 lineage during the field evaluations did not allow for proper inferences as to whether the lineage change has an effect on disease severity or the relative levels of resistance of the cultivars (**Paper IV**).

8 Future perspectives

Despite the late blight pathogen being studied for over 170 years since its discovery in Europe, very few *P. infestans* studies in Africa exist. As a consequence, many countries in Africa have no data on what pathogen lineages exist in their countries. In addition, the countries have undeveloped seed systems and a lot of external seed tuber importation occurs. Seed tuber trade is the main mechanism by which *P. infestans* is moved around the world. African countries import mostly from Europe, a continent that harbours very diverse *P. infestans* populations. If new *P. infestans* strains reach the African countries that do not focus on pathogen studies, the new strains thrive undetected. The effects of introduction of aggressive pathogen strains may be felt as occurrences of severe epidemics. This could be the situation in Nigeria where numerous reports of recent severe late blight attacks in growers' fields have surfaced (Emmanuel Nnadi, *personal communication*). The epidemics were later associated with the European 33_A2 lineage (David Cooke, *personal communication*). The appearance of the 33_A2 genotype in west Africa could be an enabler to future sexual reproduction of *P. infestans* in Africa.

There is need to assess effectiveness of fungicides currently used against the new *P. infestans* genotypes. The 2_A1 is aggressive but other fitness traits it possesses needs to be determined. As reported, severe stem blight is common in the field in eastern-Africa. This should be investigated if it is a fungicide avoidance trait occurring for specific active compounds. Moreover, the fungicide resistance spectrum of the 2_A1 genotypes need to be determined to make an informed decision as to which fungicides are effective in its management.

It is important to monitor the possibilities of the US-1 tomato-adapted genotypes eventually getting completely displaced by 2_A1 genotypes in all the countries in eastern-Africa. Also, it should be determined if the 2_A1 genotypes on potato and tomato are phenotypically different or host-specificity exists for 2_A1 genotypes isolated from potato and tomato. These are many of the

questions that need to be answered to determine whether tomato plants may act as source of inoculum for potato growers; information that lead to recommendations such as implementing disease control on volunteer tomato plants.

The possibility of *P. infestans* overcoming host resistance in cultivars with gene pyramids needs continuous monitoring. Effector genes studies should continue to aid in early detection of pathogen race variants that could overcome the resistance genes if and when they are eventually deployed. Moreover, quantifying potato cultivars, hopefully within each country should be carried out when a complete shift in the pathogen population happens to determine the existing levels of resistance. Even though most potato cultivars are grown in all countries, some unique ones are found in individual countries especially with the current acceptance of certain European cultivars which are finding their way into local seed systems.

9 Author's concluding remarks

Crop disease management in eastern-Africa can and should succeed if deliberate and thought-out actions are formulated in a clearly defined impact pathway. The aim of the impact pathway is to have more coordination as well as use of internal resources in managing crop health instead of merely importing developed technologies. To effectively apply the science generated to real time growers' issues, a number of stakeholders must work together.

Departments of agriculture: Instead of having agricultural policies as negotiated agreements, the policies should be based on widely shared scientific knowledge and growers' concerns. This affects mainly the seed trade where experiences are that there has been importation of disease-susceptible crop cultivars since the overall aim was looking for other agronomic traits in those crops. Consequently, growers are left with the burden of managing crop diseases.

International research organizations / NGOs: They should make efforts to transfer the high-end research into sustainable farming practices. Such efforts include letting the growers assess their own situations and voluntarily agree to support the sustainable agriculture. Information should be availed to growers via farmer field schools to allow for interaction and feedbacks on different plant health issues. Growers could be the first people to identify a new invasive species if they have prior knowledge on what exists.

Agrochemical companies: Companies should be mandated to allow for product performance feedback. This should be followed by withdrawal of products that are no longer effective. It has been observed over the years that companies still sell non-functional chemicals to uninformed growers hence making profits at the expense of growers. Unfortunately, the Agro-chemical industry is not regulated. Scientists need to provide hard evidence to policy makers on the inefficiency of some products to then allow for trade cessation.

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Popular science summary

Since the introduction of potato in eastern-Africa by the British farmers in the 1880s, the crop has become an important staple food as well as a source of income to the growers. Nearly all potatoes in eastern-Africa are consumed locally with rural people selling the surplus to the urban-dwellers. Also, trading with immediate neighbouring countries does happen. Seed multipliers can obtain profits upwards of 1,500 US dollars per hectare signifying the importance of the crop in the region. More than 5.6 million farmers grow potato on approximately 1.3 million hectares in sub-Saharan Africa. However, the yields obtained average between 6-10 tonnes/hectare although the projected yields can reach up to 20-30 tonnes/hectare. When it comes to tomatoes, they are grown for home consumption in the backyard of almost every homestead across sub-Saharan Africa. Tomatoes are an important cash crop for both smallholders and medium-scale commercial growers, but their yields are also generally far below the potential of the crop. A number of challenges come in the way of achieving the realistic yields for both crops and these include limited access to cultivars with good agronomic traits, low quality seed, minimal knowledge of good agricultural practices as well as pests and diseases.

Late blight is one of the diseases heavily affecting potato and tomato production in eastern-Africa. The disease is caused by the pathogen called *Phytophthora infestans* and it occurs wherever potatoes and tomatoes are grown. The disease thrives in cool and wet environments and it has been estimated to cause losses of about 13 billion US dollars per year. These losses are as a result of direct crop losses due to damages by the disease as well as the indirect costs of fungicides applications. Late blight is managed mainly by use of fungicides but use of host cultivars that are resistant to the disease could greatly reduce the cost of production. To be able to know what fungicides or plant cultivars are appropriate to manage late blight in the region, a good understanding of the pathogen population is paramount. Continuous surveillance of the pathogen

characteristics on potato and tomato will provide information that can be used to design and implement control strategies suited for the local pathogen population. Globally, *P. infestans* populations are different but similar chemical control or even host resistance genes have been used to manage the dissimilar pathogen populations.

In eastern-Africa, the late blight pathogen has not been studied extensively, but reports do indicate that a new type of *P. infestans* from Europe, named 2_A1, has outcompeted the older *P. infestans* type called US-1. The new 2_A1 had only been reported in Kenya in 2007 but in 2011, it was found in Uganda in the districts bordering Kenya. The older US-1 was the only type attacking potato and tomato in eastern-Africa before the arrival of the 2_A1. The US-1 type of *P. infestans* had subtypes attacking potato and tomato. The subtype on potato could not infect tomato in the field and vice versa. Armed with the knowledge that a new type of *P. infestans* was taking over the population in the region, we studied different aspects of the *P. infestans* population in the region. We began investigating the pathogen population in Kenya, Uganda, Tanzania, Burundi and Rwanda to determine the extent to which the 2_1A type had spread in the region. It was found that the 2_A1 type was dominating the *P. infestans* population in all the five countries. While all the *P. infestans* types on potato in Kenya were 2_A1, the old US-1 subtype on potato was still present in low numbers in Uganda, Tanzania, Rwanda and Burundi. The US-1 subtype on tomato was still present in all countries but in Kenya, both US-1 and 2_A1 were found co-existing on tomato plants. The 2_A1 isolates on tomato were more numerous than US-1 indicating the rapid displacement of the US-1 tomato subtype by the new 2_A1 specifically in Kenya. We also wanted to find out some of the characteristics the 2_A1 type had that likely enabled it to displace the US-1. Greater aggressiveness of the 2_A1 over US-1 was found to be one of the characteristics that allowed the rapid displacement of the US-1 in the region.

Genetically modified potatoes with three resistance genes, that were all put together, have been tested in the field in Uganda. For the four seasons they were in the field, the potatoes did not to get late blight attacks. The potatoes are planned to be grown in the different eastern-Africa countries in the future. For a potato resistance gene to provide protection against the invading pathogen, it must recognize pathogen genes, called effectors. Effectors are protein molecules that *P. infestans* produces to manipulate the potato so that it can invade the potato and cause disease. Sometimes these pathogen proteins are in a form that can be recognized by known potato receptors, called the resistance genes. When the pathogen proteins are recognized by the resistance genes, we call them avirulent proteins. The avirulent proteins and plant resistance genes behave like a key-and-lock system. When this happens, disease does not occur. If the form of the

avirulent proteins changes and does not match the lock-and-key system, disease results. At this point, we say the proteins are virulent proteins. Disease can also happen if the avirulent proteins are missing. We thus tested the *P. infestans* population in Kenya, Uganda, Rwanda and Burundi to see if the avirulent proteins that the genetically modified potato match to were present. We also checked for presence of the virulent proteins. The information would help to assess if the genetically modified potato would remain disease free if and when it is grown in the different countries. Results showed that the types of *P. infestans* in the region had avirulent proteins that matched the resistance genes in the genetically modified potato. Therefore, if the potato is grown in the different countries, it would not get affected by late blight disease for now. However, *P. infestans* types that have virulent proteins that do not match the potato genes might appear in future. Therefore, the best thing is to keep testing the pathogen types and subtypes present in the region to know early enough when this happens.

A European potato cultivar, Sarpo Mira, was also tested in Uganda and Kenya for its ability to adapt and retain its agronomic characteristics when grown in eastern-Africa conditions. During the field trials, cultivar Sarpo Mira, which has five resistance genes, was not attacked by late blight and is thus considered to have long-lasting resistance to late blight disease. Pathogen proteins that match two of the five resistance genes in cv. Sarpo Mira, were examined. This was to find out if they were avirulent proteins (full length protein sequences) that allowed the Sarpo Mira resistance genes to match in the lock-and-key style. The two pathogen proteins tested were AVR4 (matching the *R4* gene in Sarpo Mira potato gene) and AVR8 (matching the *R8*). Results showed that the AVR8 sequences were full length proteins hence perfectly matched the Sarpo Mira *R8* potato gene to prevent late blight development. However, the AVR4 protein sequences were short as they had some parts deleted. This means the AVR4 could not be recognized by the Sarpo Mira *R4* potato gene. Therefore, if the *R4* gene was on its own in a potato cultivar, it cannot stop late blight development. It thus seems that even if *R4* is one of the potato gene in cultivar Sarpo Mira, it was not contributing to the observed late blight resistance in the field. Any potato cultivars with only R4 gene should not be grown in eastern-Africa as they will get diseased. The *R8* gene can be transferred to other potato cultivars grown in eastern-Africa to provide protection against late blight. But also, the *P. infestans* population need to be tested continually to look out for pathogen types and subtypes that could defeat the *R8* resistance gene.

The potato growers in eastern-Africa, just like other growers elsewhere, have some popular potato cultivars that they grow in large scale, due to their market demand. Unfortunately, these cultivars get very severe late blight disease attacks

which sometimes finish the plants completely if chemicals are not used. An assessment of the commonly grown potato cultivars in eastern-Africa to determine their level of resistance to late blight disease in the field was carried out. It was found that all cultivars tested had some resistance to the late blight pathogen. Unfortunately, many of these cultivars with good resistance to late blight disease have other characteristics that growers do not like. Some do not have good taste while others take a long time in the field to mature. These are some of the explanations growers use to justify growing cultivars that easily get attacked by late blight disease. The findings from this study do indicate that late blight disease management practices and estimating the usefulness of host resistance to disease, should be made to match the characteristics of the *P. infestans* types and subtypes for the specific area.

Acknowledgements

I am grateful to so many people who have contributed greatly to seeing me succeed in completing this long doctoral journey.

First, I would like to thank Dr. Gregory Forbes who recently retired from the International Potato Center (CIP). It's through you that I got introduced to the fascinating world of *Phytophthora* research. I appreciate your patience in teaching me the very basic yet so important thing in late blight research, isolation of the *P. infestans* pathogen. You took a long trip to Uganda when I was based there and sat with me in the laboratory showing me how to do the isolations. A skill I have managed to transfer to others. Throughout my research and more so when you were my supervisor at CIP, you constantly checked on me to ensure that even though we were practically operating in different continents, all was well. You also introduced me to many people in different late blight networks as well as to the SLU group where I eventually got registered as a PhD student. Your support when I needed it most is highly appreciated.

Many thanks to Professor Jonathan Yuen for accepting my arrangements to be a PhD student in the department as I conducted my research in east Africa. Your support during my entire research and the stays whenever I visited Uppsala are greatly appreciated. Thanks for enduring the long trips you made to Uganda and Kenya to check on my progress. Even though you retired before my doctoral journey was over, you ensured all was okay when you were my main supervisor and even after. To my main supervisor Dr. Malin Elfstrand, many thanks for making sure the very important requirements I needed to fulfil as a doctoral student were done in the shortest time possible. Thanks for the encouragements too and giving positive criticism to my write-ups. To my supervisor Dr. Björn Andersson, thank you so much for the timely responses to many research questions that I posed to you especially when conducting my phenotypic studies. Your help throughout the research journey while I was in east Africa and in Uppsala is invaluable. To Dr. Annika Djurle, thanks for the personal care whenever I visited Uppsala. Thanks to the other people at the department whom

I interacted with. A special mention goes to Dr. Lina Sjöhlm and Dr. Anna Berlin whose friendship, while in Uppsala and away, means a lot to date.

I would like to express my gratitude to the International potato center (CIP) for allowing me to enrol as a PhD student in Sweden while still working for the organization in sub-Saharan Africa. Many thanks go Dr. Marc Ghislain whose positive criticism while conducting the research and writing this thesis helped me a lot. Your support even outside the research work is appreciated. My supervisor Dr. Jorge Andrade, thank you for all the support. To all the other past and present colleagues in CIP-sub Saharan Africa who helped me in one way or another to achieve the different objectives of this research, your help is greatly appreciated. A special mention goes to Dr. Rogers Kakuhenzire who helped me with designing field sampling plans in Uganda and for also agreeing to personally do the field sampling of *P. infestans* leaf samples in Tanzania.

Many thanks to the staff at Uganda's Kachwekano Zonal Agricultural Research & Development Institute (KaZARDI) for their support when I was hosted by NARO as a CIP staff. A unique mention goes to Elizabeth Natukunda (RIP), you were my guide in all the field trips we made in the different districts of Uganda as well as my research assistant in the lab. You died so young, eternal rest to your soul my friend.

Many thanks to the BecA-ILRI Hub for contributing greatly to my PhD research through a research fellowship enabling me to conduct the research in Kenya. Special thanks to Dr. Wellington Ekaya and Dr. Roger Pelle for facilitating my fellowship application. Thanks to Valerian for handling issues with a lot of kindness. To Collins Mutai, thanks a lot for all the assistance in the lab. You all ensured all was well during and after the fellowship. Asanteni sana!

I would like to thank Dr. Alison Lees and Dr. David Cooke of the James Hutton Institute (JHI) and the Euroblight netwok as well as Dr. Jean Ristaino and Amanda Savile of North Carolina State University (NCSU) for the help with *P. infestans* DNA reference samples, microsatellite reference data as well as laboratory protocols. All your help is greatly appreciated.

Last but not the least is my family and friends. Most of you have no idea what I have been working with and to you I am just a scientist. Nonetheless, your support has been invaluable all these years. To my husband Joab and, daughters Cindy and Lisa, you have always braved my numerous absences but also were in agreement that mommy had to finish her "home work of four years" because the writing journey never stopped even at home. I love you all!

This research was undertaken as part of, and funded by, the CGIAR Research Program on Roots, Tubers and Bananas RTB and supported by CGIAR Fund Donors. The laboratory aspects of this work was funded by the BecA-ILRI Hub through the Africa Biosciences Challenge Fund ABCF program.