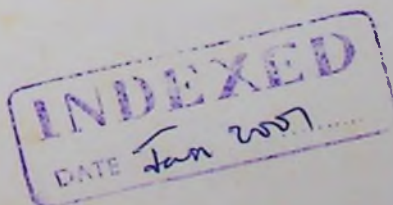


VARIABILITY IN LATE BLIGHT SEVERITY AND PATHOGEN POPULATION IN UGANDA

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B.Sc. (Agric.) Hons.

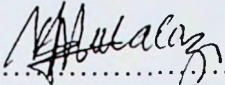
A thesis submitted in partial fulfilment of the requirements for the Degree of Masters of
Science in Agriculture (Crop Science) of Makerere University

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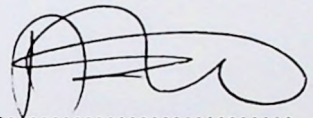
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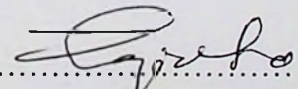

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DEDICATION

To my family: Jackline, Oscar and Edgar

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SUMMARY

In Uganda, it is suspected that late blight (*Phytophthora infestans*) is largely responsible for the low tuber yields of potato. However, there is no quantitative information on the variability in occurrence of late blight disease in the country, yield losses due to the disease, and little is known about the population structure of *P. infestans*. This study was therefore, undertaken to: a) establish the variability in occurrence of late blight disease and yield losses due to the disease in major potato growing areas of Uganda, and b) characterise *P. infestans* populations in Uganda with respect to occurrence of A2 mating type, pathogen resistance to metalaxyl, and cross infection between potato and tomato.

The first part of the study involved field surveys in the districts of Kisoro, Kabale, Mbarara and Mbale. Potato fields were assessed for late blight incidence and severity. Information on type of fungicide used, number of sprays, and names of cultivars were also noted. Yield loss assessments were done in farmers fields in the districts of Kabale and Mbarara during the second season of 1998.

Late blight incidence and severity varied significantly ($P \leq 0.05$) from district to district and season to season during the four surveys. Overall, Kabale district consistently recorded the highest late blight severities, with mean severity of 27.6%, followed by Mbale (21.8%), and Mbarara the least severity (10.8%). There was more disease during December 1997, with mean severity of 40.2%, while the least severity was in May 1998. The highest severity was recorded on variety Victoria (62.1%) followed by Wafrika (61.8%). Sangema (59.5%), Maboni (48.2%). Nevertheless, there was only a weak

negative correlation between late blight severity and yield of potatoes, likely because the yield loss assessment was done during a season of low late blight occurrence.

Eighty one *P. infestans* isolates recovered from potato samples from Kisoro, Kabale, Mbarara and Mbale were tested for their sensitivity to metalaxyl. Overall 44.4% (36 of 81) of the isolates tested were resistant to metalaxyl, 23.5% intermediate and 31.2% sensitive. In the mating type test, using a known A1 (1724) isolate, 50.0 % of the isolates produced oospores in matings and selfings, 22.5% in mating only and 10% in selfing only; 18% did not produce oospores in the two tests. A total of 27 (44.2%) of the 61 *P. infestans* isolates from potato infected tomato leaves of which, the majority were highly resistant to metalaxyl and produced oospores in either mating or selfing or both.

The existence of metalaxyl resistant isolates is an indication of the possible existence of new *P. infestans* population in Uganda. Most of the metalaxyl resistant isolates were from those districts using the fungicide ridomil. The presence of oospores in cultures suggests the potential for hybridisation and selfing of *P. infestans* in potato growing areas of Uganda, but does not confirm the existence of A2 mating type. The isolates need to be crossed with A2 tester isolate, and isozyme and DNA fingerprinting are needed to confirm the presence of the A2 mating type.

CHAPTER ONE

INTRODUCTION

1.1 Importance of potato

The potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world after wheat, rice and maize (Fehr, 1987). It ranks first among root and stem tuber crops and plantains (Burton, 1966) and accounts for 52.9% of world's production of all root and tuber crops (Simmonds, 1971; Anonymous, 1990). With increasing human population on limited land area, production of carbohydrates and proteins per unit area must be stepped up. The potato is able to contribute to this because it gives yields in a short growing season (70-120 days) (Rhoades, 1982; Horton, 1987).

Potato production is on an increase in most developing countries, but yields per unit area are still low. In Uganda, the average national yield of 6.4 tons/ha (FAO, 1999) are far from the potential. At Kalengyere Research Station yields of 25 t/ha have been attained (Hakiza *et al.*, 1997b), and farmers around this station realise up to 20 t/ha (Low , 1997). In other parts of the country, however, yields rarely exceed 10 t/ha (Adipala, 1999). Nevertheless, potato is becoming an increasingly important food and cash crop in Uganda. The per capita consumption per annum is 19.1 kg, but is more than 100 kg in South-western Uganda, the major potato producing region of the country (Ewell, 1991). Most of the crop is produced at subsistence level on small farm holding of 2 to 4 hectares (Adipala, 1999)

1.2 Potato production in Uganda

The potato was introduced by colonial administrators around 1900 (Akimanzi, 1982). It gradually gained importance as a food crop in the highlands of Uganda (Kabale, Kisoro and Mbale districts). European varieties were introduced, and they were maintained for decades. With expanded production, late blight (*Phytophthora infestans* (Mont.) de Bary) increasingly became a problem in the highlands. In response to late blight and other production problems, Uganda initiated a Potato Research and Development Program in 1968 under the then Ministry of Agriculture in collaboration with Makerere University, USA and later the International Potato Centre (CIP). By 1974, eleven varieties of Mexican origin including Uganda 11 (Rutuku), Uganda 16 (Rosita), Uganda 22 (Makerere) and Uganda 36 (Wurster) were released in Kabale and Kisoro districts (Akimanzi, 1982). Some of these varieties, such as Rutuku, remain very popular today.

Additional releases of three varieties (Victoria, Kisoro, Kabale) were made in 1991 (Sikka, 1994), and in 1999 three more varieties, namely Nakpot 1 (CIP 382171.4), Nakpot 2 (CIP 381403.8) and Nakpot 3 (575049) were released by the National Potato Program (Anonymous, 1999). In addition to local releases, introductions were made from Rwanda National Potato Program (PNAP) through PRAPACE in 1987. Some of Rwanda varieties now being grown include Sangema and Cruza-148.

Uganda is characterised by a wide range of diverse cropping systems, but in the highland elevations (> 1500 masl) potato based cropping system predominate. The areas have two growing seasons in a year. Potato on a few occasions is intercropped with beans (*Phaseolus vulgaris* L), peas (*Pisum sativa*), maize (*Zea mays* L) or sorghum (*Sorghum*

bicolor). Because of land shortage in the highlands rotation is not well practiced. In these areas, potato is both a staple and cash crop (Sikka, 1994). The highland areas of western Uganda (Kabale, Kisoro, Mbarara, Kasese, Kabarole), eastern (Mbale, Kapchorwa) and north-western (Nebbi, Arua) are the best potato growing areas in the country (Sikka *et al.*, 1991). The climatic conditions (15-17°C and > 1200mm of rainfall per annum) favour production of high quality seed and high yields, comparable to in temperate areas (Sikka *et al.*, 1997). It is also possible to grow potatoes in low-warm environments (Hakiza *et al.*, 1997a). Thus, potato is currently being grown in areas like Mityana, Masaka and Rakai. Although the cool climate through out the year and the well-distributed rainfall favour potato growing, unfortunately, these same conditions are conducive to the rapid build-up of pests and diseases in the soil and seed (Rowe, 1993).

1.3 Potato production constraints

While potato hectareage has increased in Uganda in recent years, from 7000 ha in 1966 (Mukiibi, 1972), to 24,000 ha in 1980 and to 56,000 ha in 1998 (FAO, 1999), the production per unit area at farm level has remained below 10 ton/ha (Adipala, 1999; FAO, 1999). The low yields are attributed to a multitude of factors. Late blight (*Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum* E.M. Smith) and limited availability of improved seed are the major constraints to successful potato cultivation. In some instances repeated use of the same seed stocks has resulted in devastating disease epidemics, drastically reducing yields (Low, 1997). Late blight significantly reduces potato yields and takes a large proportion of potato production cost through purchase of fungicides and the added labour cost for spraying (Anonymous, 1998). Bacterial wilt

causes an estimated yield loss of over 30% in Uganda (Alacho and Akimanzi, 1993), and occasional losses of up to 100% have been reported (Opio, 1988; Kakuhenzire *et al.*, 1993). Potato viral diseases are common wherever the potato is grown (Mendoza, 1993). However, their range and status vary with location. In Uganda, potato leaf roll virus (PLRV), potato virus X (PVX) and potato virus Y (PVY) are the main causes of crop degeneration (Kakuhenzire, 1996).

1.4 Justification of the study

Phytophthora infestans (Mont.) de Bary, the causal agent of late blight of potato and other solanaceous plants, is heterothallic, and both mating types must normally be present for the formation of oospores. The centre of origin of *P. infestans* is in Mexico, in Toluca valley, where both mating types A1 and A2 exist in approximately equal proportions, and sexual reproduction appears to be widely spread (Tooley *et al.*, 1985). The A1 mating type was the only one that spread worldwide. The A2 mating type was first identified outside Mexico in Switzerland in 1984 (Hohl and Iselin, 1984). Subsequently, A2 isolates have been reported outside Europe (Spielman, 1991). The A2 mating type was discovered in imported Egyptian ware potatoes to UK in 1985 (Shaw *et al.*, 1985). This was an indication of the possible occurrence of A2 mating type in Africa.

Current information on population dynamics of the fungus indicate that the A2 mating type belongs to a new sexual population that carries both A1 and A2, and which probably escaped from central Mexico in the late seventies (Fry *et al.*, 1992). It has also

been shown that these new populations rapidly replace the original lineage in many regions (Fry *et al.*, 1993). These new populations seem not only to carry the potential to complete their sexual cycle but appear to have higher levels of virulence and higher frequencies of resistance against the fungicide metalaxyl (Deahl *et al.*, 1995; Goodwin *et al.*, 1996).

In Uganda the increase in land area under potato production has also been characterised by increased occurrence of devastating late blight epidemics (Anonymous, 1996). Over forty percent of potato farmers in Uganda use fungicides to control potato late blight (Anonymous, 1998). In some instances farmers have indicated that to reduce late blight severity they have to apply fungicides weekly (Adipala, 1999). In other cases fungicide application appear not effective (Anonymous, 1998). There is a need therefore to monitor carefully any build-up of resistance against the fungicide used as this can present a real economic threat to potato production in Uganda. There is no quantitative information available on the variability in occurrence of late blight disease in the country, and little is known on the present structure of the fungus in Uganda. With the existence of new strains of late blight fungus reported elsewhere, information is required on the variability in occurrence of potato late blight disease and the population structure of *P. infestans* in the country.

1.5 Objectives of the study

The present study was undertaken with the following objectives:

- a) To establish the variability in occurrence of late blight disease in major potato growing areas of Uganda.

- b) To characterise *P. infestans* populations in Uganda with respect to occurrence of A2 mating type, pathogen resistance to metalaxyl, and cross infection between potato and tomato.

The study is based on two hypotheses; 1) late blight is important in only a few places in Uganda, 2) A2 mating type is present in the country and has contributed to the development of fungicide resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1 Late blight

2.1.1 Economic importance

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, first devastated potato crops in Europe, and led to the Irish potato famine 150 years ago (Fry and Goodwin, 1997). The fungus is again creating a major problem today. Crop loss due to potato late blight increased dramatically in the early 1980's in Europe, and subsequently, in the Middle east (Fry *et al.*, 1993). In the late 1980's and early 1990 severe epidemics occurred in North America. The disease reduced marketable yield of potato by about 80%, despite doubling the use of fungicides during the period 1992 to 1994 (Fry and Goodwin, 1997).

Late blight is now so important internationally that in March 1996, the International Potato Centre in Lima, Peru, called for a global initiative to combat late blight (CIP, 1996). This has become the subject of meetings and newsletters involving scientists and growers worldwide. The problem of late blight in Africa as reviewed by Sengooba and Hakiza (1999), is associated with serious yield losses, high cost of fungicide and few late blight tolerant varieties. The problem is further complicated by breakdown of resistance in potato cultivars (Turkensteen, 1988). An integrated disease management (IDM) research strategy is being adopted to combat late blight in Uganda, and other parts of the world

(CIP, 1996). The components include; use of resistant/ tolerant varieties, use of fungicides, manipulating planting dates, sanitation and others as applicable under different cropping systems. In Eastern Africa all countries are either directly involved in late blight research activities or at least they do screening/selection from the regional trials (PRAPACE/CIP, 1996) In case of Uganda, varieties Rutuku selected in the 1970's (Akimanzi, 1982), Victoria, Kabale and Kisoro released in 1991 (Sikka, 1994), and Nakpot 1 (382171.4), Nakpot 2 (381403.8) and Nakpot 3 (575049) in 1999 (Anonymous, 1999) were chosen for their late blight tolerance among other major attributes. Selection for genotypes in populations A continues, and work on population B is on-going at Kalengyere Research Station at all times giving due attention to resistance to late blight (Hakiza *et al.*, 1999).

2.1.2 The causal organism

Late blight is caused by *Phytophthora infestans* (Mont.) de Bary, a downy mildew fungus (Dutt, 1979). The organism is placed in the family Pythiaceae, order Peronosporales of the class Oomycetes (Alexopoulos and Mims, 1979). *P. infestans* has sporangia (conidia) that are hyaline, lemon-shaped, thin-walled, and 21-38 by 12-23 μm (Hooker, 1990). The sporangia of *P. infestans* develop at the tip of a sporangiophore; as it elongates the sporangiophore swells slightly and turns the attached sporangium to the side. The sporangiophore is thus characterized by periodic swellings at points where sporangia are produced (Hooker, 1990).

Sporangia may germinate by means of a germ tube, but more commonly they form biflagellate zoospores that swim freely in water and encyst on solid surfaces. The zoospores can germinate by germ tubes that enter the host via stomata, but they usually form appressoria and penetrating hyphae which enter directly through the cuticle. Once inside the plant, the nonseptate mycelium is intercellular, and intracellular by means of haustoria that extends into cells (Hooker, 1990).

P. infestans can reproduce by sexual means where by oospores are formed by union of oogonia and anthredia. Oospores that are formed in plant leaves are aplerotic, 20 to 35 μm in diameter, in artificial culture they measure 24 to 56 μm in diameter (Erwin and Ribeiro, 1996). The fungus is heterothallic, two mating types A1 and A2 must normally be present for the formation of oospores (Tooley *et al.*, 1985). Oospores enable the fungus to survive in the soil, and in debris up to two years, hence may provide inoculum for early infection of emerging potato plants (Niederhauser, 1991).

2.1.3 Disease symptoms

Infected leaves first become yellow then water-soaked and eventually turn black. The symptoms may appear on the foliage any time during the development of the plant, containing purple or black or brownish lesions and usually appear first at the tip or margin of the leaf. Later lesions may occur anywhere on the leaf, petiole or stem. The entire vine may be killed and blakened in a short time when damp weather persists. If conditions are favourable, white mass of sporangia appear on the underside of the leaf.

Tubers get affected later in the season. In early stages, lightly brown or purple blotches appear on the skin. In damp soils, the disease progresses rapidly, and tubers decay (Stevenson, 1993).

2.1.4 Disease Development

Potato tubers not well covered by soil may be infected in the field by spores that tend to be washed from infected leaves into the soil by rain. Tuber infection may also occur during wet harvest conditions via contact between tubers and sporangia on vines or via air-borne sporangia (Hooker, 1990; Stevenson, 1993). This, therefore, enables *P. infestans* to overwinter as a mycelium in unharvested potato tubers, and tubers stored as seed. After planting, the fungus invades the sprouts under favorable conditions, producing primary inoculum (Hooker, 1990). The fungus survives on solanaceous plants, potato and tomato being the main hosts (Dutt, 1979). Sporangia are normally produced rapidly on leaves at temperature 21°C and relative humidity of about 100%. The sporangia are splashed by water or spread by wind (Hooker, 1990).

2.2 Late blight control

2.2.1 Chemical control

Application of fungicides prior to infection in the field has been one of the main approaches to control late blight (Fry *et al.*, 1979). The fungicides that have been used to control the disease include bordeaux mixture and other copper compounds that are less

toxic, pthalimides, and the dithiocarbamates (Erwin and Ribeiro, 1996). The dose, rates and application times varies with weather conditions (Schwinn, 1983). Fungicide use for the management of late blight in eastern Africa is reviewed by Sengooba and Hakiza (1999). It is highly variable among the countries and farmer categories. In the Democratic Republic of Congo fungicides are rarely used, while Burundi reports moderate usage. Most Rwanda farmers spray 3 times and Kenya 5-7 times. In Uganda about 45% of potato farmers use fungicides to control late blight, spraying on average 2 to 3 times a season (Anonymous, 1998; Adipala, 1999).

There has been improved control of late blight through use of systemic phenylamide fungicides such as metalaxyl. They are normally applied with a protectant fungicide such as mancozeb to help suppress the development of phenylamide-resistant biotypes of *P. infestans* (Schwinn, 1983). Although metalaxyl and other phenylamide fungicides are the most commonly used in the control of late blight, the development of resistance within populations of *P. infestans* has become a limiting factor in their use (Davidse *et al.*, 1981). For example, in the Netherlands, 11% of isolates from community gardens where metalaxyl had not been used were resistant, but 45% of the isolates were resistant in fields where metalaxyl had been used (Fry *et al.*, 1991). Development of resistance to metalaxyl has been alleviated to some extent by combining a broad spectrum fungicide, such as mancozeb, with metalaxyl and by limiting the number of field sprays with metalaxyl (Gisi and Cohen, 1996).

2.2.2 Host resistance

Breeding for race- specific resistance to late blight was considered to be an efficacious approach to control the disease, but has proved to be of only limited use. This is because many pathogenic races of *P. infestans* have the ability to attack cultivars with single-gene resistance (Erwin and Ribeiro 1996). Breeding efforts are now shifting towards horizontal resistance, which is not controlled by dominant resistance genes, as in race-specific resistance. This type of resistance does not confer a high level of resistance, and has been identified in many cultivars (Niederhauser, 1991). After the failure of single-gene type resistance, horizontal resistance is now the best approach to genetic control (Umaerus *et al.*, 1983). As explained by Vanderplank (1963), horizontal resistance reduces the rate of the epidemic and is equally effective against all races of the pathogen. It is more stable because it is conditioned by more than one gene and there is low probability that mutations in *P. infestans* populations will occur simultaneously in nature, in more than one gene at a time (Niederhauser, 1991).

In Uganda, there are on-going efforts to search for horizontal resistance to potato late blight at Kalengyere Research Station. In this regard, population B3 genotypes which lack R-genes and race-specific resistance, have been obtained from CIP, Lima, Peru. Some of the clones have exhibited high levels of horizontal resistance (Hakiza *et al.*, 1999)

2.3 Migration of *P. infestans* populations

In the past populations of the late blight fungus, *P. infestans*, found outside Mexico contained only one mating type, A1. This meant that except in Mexico, considered to be the origin of this pathogen (Niederhauser, 1991), the fungus could not complete its sexual life cycle which includes formation of oospores. The A2 mating type was first identified outside Mexico in Switzerland in 1984 (Hohl and Iselin, 1984). The probable mechanism of migration of the A2 mating type was via infected potato tubers. Large quantities of potatoes for domestic consumption were apparently imported from Mexico to Western Europe in the late 1970's (Niederhauser, 1991; Fry *et al.*, 1993).

A considerable amount of evidence suggests that about 20 years ago new populations of late blight fungus *P. infestans* containing both A1 and A2 mating types escaped from Mexico and started spreading around the world from Holland (Fry *et al.*, 1992). The new populations are rapidly replacing the original lineage in many regions (Fry *et al.*, 1993). They carry the potential to complete their sexual cycle and appear to have higher levels of virulence and high frequencies of resistance against the widely used fungicide metalaxyl (Deahl *et al.*, 1995; Goodwin *et al.*, 1996), which might have an important influence on the epidemiology of late blight.

The migration of the new *P. infestans* population throughout many regions of the world, the occurrence of metalaxyl resistance in that population, and the diverse mechanism of dispersal of the fungus, are pointers to possible new outbreaks of the disease (Fry *et al.*, 1993). This, therefore calls for continuously monitoring the population structure of the

fungus in terms of fungicide resistance, mating types and virulence tests on different host plants in this region.

2.4 Sexual reproduction in *P. infestans* and genetical determination of mating type

Heterothallism in *P. infestans* is a system of two mating or compatibility types, A1 and A2. Each of these mating types is self-sterile when grown in isolation but is bisexual, i.e., can form both antheridia and oogonia when suitably stimulated or self-fertilised (Shaw and Shattoch, 1991). Mating types of isolates of heterothallic species of *Phytophthora* are determined by pairing with opposite mating types of *P. infestans*. Pheromone which stimulate sexual activity reciprocally tend to be released by a pair of compatible thalli (Ko, 1988). Progenies of *P. infestans* from matings segregate to give varying proportions of A1 and A2 mating types which often deviate from a 1:1 ratio. Backcross and F₂ progenies tend to deviate more than F₁ progenies (Shattock *et al.*, 1987). A few self-fertile progeny are often observed which usually revert to A1 or A2 during vegetative growth. Indeed sporangia from a single germinating spore can give rise to both A1 and A2 zoospore lines (Laviola and Gallegly, 1983).

Some information available support the fact that one mating type is a simple heterozygote for a mating type gene. However, selfs of *P. nicotianae* produced in a similar manner showed segregation of A2 from A1 parents and segregation of A1 from A2 parents (Ko, 1988). In *P. nicotianae* species, growth of a single mating type of the pathogen in presence of certain fungicides can result in self-fertile cultures from lines of A1 and A2

mating type which can be isolated. For example, Chloroneb fungicide induces A1 in A2 cultures whereas Truban induces A2 in A1 cultures (Ko, 1988). Similarly, fungicides may be inducing changes in *P. infestans* populations which might be the case for this region.

2.5 Pathogen resistance to phenylamide fungicides

The systemic organic fungicide metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester] is commonly used to control late blight of potato and tomato. Metalaxyl inhibits vegetative growth as well as sexual and asexual reproduction of many isolates of *P. infestans* (Cohen and Coffey, 1986). Resistance to metalaxyl, with resulting failure to control late blight, has been problematic since the fungicide was first widely applied (Davidse *et al.*, 1981; Dowley and O'Sullivan, 1981). Metalaxyl resistance is common in *P. infestans* populations and is found in isolates of different pathogenic specificities and mating types (Deahl *et al.*, 1991; Fry *et al.*, 1992). Some isolates have been reported to grow better in cultures when metalaxyl was incorporated in the medium than when it was omitted (Zhang *et al.*, 1997).

Metalaxyl inhibits RNA synthesis through inhibition of RNA polymerase I and /or RNA polymerase III (Davidse *et al.*, 1983b). In case of metalaxyl resistant mutants, RNA polymerase I /or RNA polymerase III is the target site for metalaxyl and resistance is the alteration of the target site (Davidse *et al.*, 1983b). High frequencies of metalaxyl resistant *P. infestans* isolates have been reported in areas where metalaxyl alone is widely used to control late blight and also where low dosage is used (Davidse *et al.*, 1981;

Dowley and O'Sullivan, 1981; Davidse *et al.*, 1983a). With attempts to reduce metalaxyl resistant *P. infestans* genotypes, the strategies include use of prepacked mixtures of metalaxyl with well defined rates of nonphenylamide fungicides (Gisi and Cohen, 1996), and limitation of number of application per crop. The commercial product available in Uganda, Ridomil MZ 63.5 WP contains 7.5% metalaxyl and 56% mancozeb (Ciba-Geigy, 1996), which reduces chances of developing resistant *P. infestans* genotypes.

2.6 Inheritance of fungicide resistance

Phytophthora infestans phenotypes resistant to phenylamide were first detected soon after commercial release of metalaxyl (Davidse *et al.*, 1981; Dowley and O'sullivan, 1981), their frequencies have since increased in numerous countries. Shattock (1988) suggested that a single incompletely dominant gene was involved in the inheritance of resistance to metalaxyl, although backcross progeny showed unexpected frequencies of phenotypes, isozymes, and sensitivity to metalaxyl (Shaw and Shattock, 1991). Resistance to metalaxyl, as suggested by Kadish and Cohen (1988), is controlled by a single gene codominant nuclear locus, and single deviation in F_1 progeny probably result from selfing. The possible linkage between the locus for metalaxyl resistance and other loci, eg., A2 mating type, probably enhanced fitness, polyploidy, and complex virulence (Drenth *et al.* 1993; Ko, 1994). The frequency of resistance to metalaxyl in both matings was reported to be equal in isolates from Netherlands (Therrien *et al.*, 1989). They proposed that, the initial mutation for metalaxyl resistance in Netherlands occurred in A1 and later A2, either spontaneously or via recombination with A1. Probably this same situation may be occurring in the late blight pathogen population in eastern Africa.

2.7 Host specificity of *P. infestans*

In Ecuador, isolates from potato infect potato more aggressively than tomato (Forbes *et al.*, 1996). Contrastingly, tomato aggressive isolates cause severe disease on both potato and tomato, but significantly more disease on tomato than potato (Legard *et al.*, 1995). On the other hand, the tomato-non aggressive *P. infestans* isolates have been found to cause significantly less disease on tomato than potato (Forbes *et al.*, 1996). It appears, therefore, that tomatoes could be grown in nearby potato fields without late blight infection to potato.

In Netherlands, some isolates of *P. infestans* recently recovered from tomato constituted a population genetically distinct from potato isolates (Fry *et al.*, 1991), and at another site the genotype found on tomato was the predominant genotype recovered from potato. Recent work in the USA has found that certain genotypes are recovered from both potato and tomato whereas other genotypes are only recovered from potato (Goodwin *et al.*, 1994; Goodwin *et al.*, 1995). This suggests that a single genotype of the pathogen can be predominant in a given location and may cause disease to different host species. Kori (1972) pointed out that in East Africa, *P. infestans* was recovered from some wild woody perennial species of *Solanum*, namely *S. indicum* L., *S. incunum* L., and *S. aculeastrum* Duna. Nattrass and Ryan (1951) point out that, sporangia from *S. incunum* readily infected potato, and in turn, sporangia from these infected potatoes reinfected *S. incunum*.

2.8 Late blight occurrence and yield loss

Late blight is one of the several diseases that attack potato crops. The disease inflicts damage on field foliage and storage tubers (Fontem and Aighewi, 1993). Disease incidence and severity vary yearly and are governed mainly by weather conditions (Callbeck, 1960). Additionally, the occurrence and economic importance of potato late blight during the last 10 years or so has been influenced by the migration and/or development of new pathotypes (both A1 and A2 mating types) in various potato producing areas. For example, in Columbia Basin, the increased occurrence of late blight resulted in 4 to 5 % reduction in harvested yields and almost \$ 1.3 million loss in additional disease management costs (Johnson *et al.*, 1997).

Studies of crop losses have gained increased attention and the agricultural and economic rationales for evaluating them have been described (James, 1974). Different methods have been employed for the evaluation of losses in different crops and diseases (James, 1974), and same crops and diseases in different countries, as in the case of potato late blight (James *et al.*, 1972). Each method was to find a relationship between disease severity and yield loss. In some reports the yield, in different crops, was expressed in weight measured (Nelson *et al.*, 1976; Young and Ross, 1979), but in most reports yield loss was expressed as a percentage derived by comparison with the healthiest plot (James *et al.*, 1972; Lim, 1980). Unfortunately, there has been limited information about variation in yields. Also, loss in yield is usually analysed in relation to percentage defoliation of host plant. However, the plant size may differ in different seasons and

locations. Therefore, with the same percentage defoliation, different amounts of healthy foliage may persist (Rotem *et al.*, 1983) and affect yield differently.

Yield loss studies in Cameroon indicate that potato late blight epidemic rate and area under disease progress curve (AUDPC) were significantly reduced in sprayed plots while potato yield increased considerably (Fontem and Aighewin, 1993). Yield losses associated with non-sprayed plots in Cameroon were as high as 50% and 70% for varieties Dschang and Bansao, respectively (Fontem and Aighewin, 1993). Relative yield loss at farmers level and the contribution of late blight to the loss are, however, not well documented. As reviewed by Sengooba and Hakiza (1999), farmers in eastern Africa perceive late blight as a problem causing economic loss as much as over 90%, if the disease strikes a susceptible variety early in the growing season. In Uganda, yield losses from late blight are estimated at 40-50% for a moderate resistant varieties, and 50-70% for the susceptible varieties (Anonymous, 1996). In Kenya, yield losses were estimated to vary between 56-76% (Lunga'ho, 1998). In the case of Ethiopia, tuber yield losses of 67 and 45% on a susceptible variety AL-624 have been reported, but only 15 and 3% yield loss on the tolerant variety K-59A (26) (Bekele and Yaynu, 1996).

2.9 Section conclusion

From the above literature review, some of the major the gaps that require further investigations are; (a) to document information on the variability in late blight occurrence in potato growing areas of eastern Africa, specifically, in Uganda, (b)

establish yield losses caused by potato late blight at farmer level, since the available information is based on on-station data, and (c) characterise population structure of late blight fungus in Uganda with respect to presence of A2 mating type, fungicide resistance and cross infection of potato and tomato isolates.

CHAPTER THREE

LATE BLIGHT OCCURRENCE IN UGANDA

3.1 Introduction

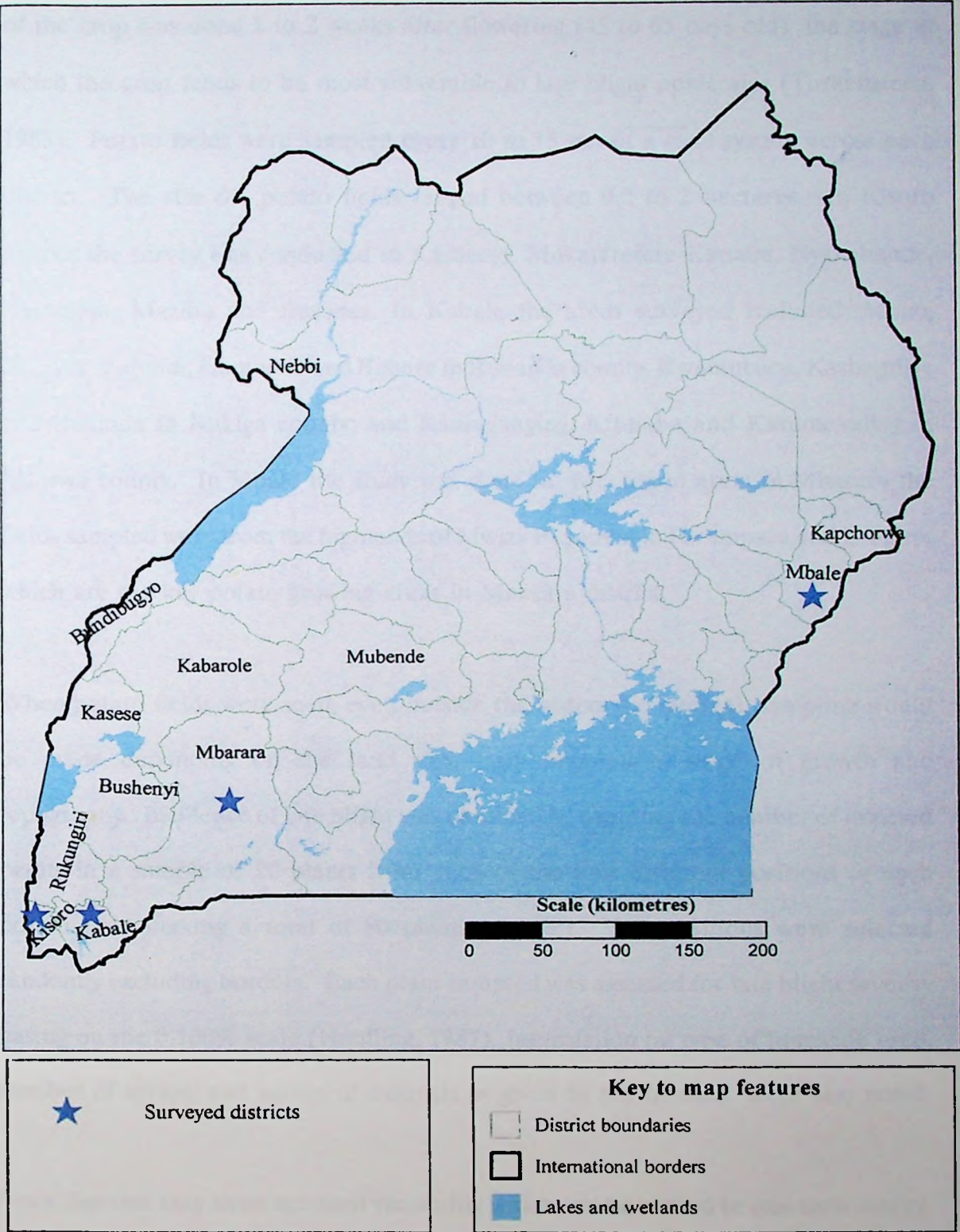
Late blight caused by *Phytophthora infestans* (Mont.) de Bary is probably the most limiting disease to potato production in eastern and central Africa (CIP/PRAPACE, 1996). In Uganda, late blight is endemic and destructive in highland areas, although occasional epidemics occur in lowland areas (Bhagsari *et al.*, 1994). If the disease is not controlled, yield losses can reach up to 100% (Stevenson, 1993). While the above may be true, information on the actual variability in occurrence of late blight and yield loss attributable to the disease on potato in Uganda are not known. This study was undertaken to establish the incidence, severity and damage of late blight in two major potato growing agroecologies of Uganda.

3.2 Materials and methods

3.2.1 Study area and disease assessment

Field surveys were carried out in south western Uganda, covering the districts of Kisoro, Kabale, and Mbarara, and in Mbale district in eastern Uganda (Fig.1). These form the major potato growing areas of the country (Sikka *et al.*, 1991).

Fig. 1: Major potato growing districts of Uganda



In each district, the number of potato fields sampled varied from 10 to 35. Sampling of the crop was done 1 to 2 weeks after flowering (45 to 65 days old), the stage at which the crop tends to be most vulnerable to late blight epidemics (Turkensteen, 1988). Potato fields were sampled every 10 to 15 km of a road system across each district. The size of potato fields ranged between 0.2 to 2 hectares. In Kisoro district the survey was conducted in Kashenyi, Mukateretere-Kanaba, Nyakabande, Nyarusiza, Maziba and Busanza. In Kabale the areas surveyed included: Muko, Ikumba, Bufundi, Hamurwa and Bubare in Rubanda county; Rwamucucu, Kashambya and Bukinda in Rukiga county; and Kamuganguzi, Kitumba and Katuna valley in Ndorwa county. In Mbale the study was done in Mt. Elgon area; in Mbarara the fields sampled were from the highlands of Mwizi-Bugamba in Rwampara and Isingiro, which are the key potato growing areas in Mbarara district.

When potato fields were seen, even outside the systematic interval, sampling would be made depending on the land size, crop population, stage of growth and topography. Incidence of late blight was obtained by counting the number of infected plants in a sample of 20 plants from each of the four different positions of each potato field making a total of 80 plants per plot. The positions were selected randomly excluding borders. Each plant sampled was assessed for late blight severity basing on the 0-100% scale (Henfling, 1987). Information on type of fungicide used, number of sprays, and names of cultivars as given by the farmers were also noted.

Since diseases may show seasonal variability which can be missed in one-time survey, the survey was done four times, i.e., December 1997, May 1998, November 1998 and

May 1999. Sampling in the different seasons was done in the same villages, although not necessarily from the same farmers. Seasons and districts were considered as treatments and fields within each district were the replications. Incidence and severity data for all seasons were transformed using arcsine transformation $(x+1)$, to harmonise variance and pooled together prior to Analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS statistical package (SAS, 1987). Means (transformed) were separated by Least Square means (Searle *et al.*, 1980).

3.2.2 Assessment of yield loss due to late blight

For logistical reasons, yield loss assessments were only done in farmers fields in the districts of Kabale and Mbarara during the second season of 1998. Farmers were selected from the sub counties of Muko and Bubale in Kabale district and Mwizi in Mbarara district. Potatoes are intensively grown in these areas and late blight is a major production constraint.

Twelve farmers' potato fields (plots) were sampled during the study, 6 farmers from Muko and Bubale in Kabale district, and 6 were from Mwizi sub-county in Mbarara district. The fields were assessed three times, the first during the second week of November 1998 (vegetative stage), the second during the first week of December 1998 (flowering stage) and the last one in the first week of January 1999 (mature stage).

During each visit, severity of late blight disease was recorded from 3 randomly selected positions within each potato field as described earlier. Field characteristics and management practices were noted at each field visit. On average, each sampling position measured 5 m x 1.4 m and were considered replications for data collection. At harvest the same sampling procedure was used. The severity of late blight infection of tubers was also evaluated at the time of harvest. Potatoes were harvested at 3 different positions in each field, and the average tuber weight (kg) recorded. After harvest, 20 tubers were randomly selected from each of the three sampled portions of each plot, and the level of tuber late blight attack as well as other pests and diseases assessed. The data generated were subjected to Analysis of variance (ANOVA) using general linear model (GLM) procedure. Additionally, late blight severities were related to tuber yields by correlation and regression analyses. All statistical analyses were done using SAS Statistical Package (SAS, 1987).

3.3 Results

3.3.1 Potato varieties grown in Uganda

Different districts were found to grow different potato varieties as named by farmers, and some varieties were more common than others. Most of the varieties encountered bore the names and characteristics of those released varieties in Uganda. Except two varieties *Maboni* and *Wafurika* found in Mbale. Overall, Victoria was the most commonly grown variety in all the four districts, whereas Musitamya was common in the three districts except Mbale. Kabale district had the highest number of varieties grown which include;

Cruza, Kabale, Kisoro, Malirahinda, Mbumbamagara, Musitamya, Rutuku, Sangema, Victoria and Nakpot 1 (382171.4). Kisoro district had; Cruza, Kabale, Mbumbamagara, Musitamya, Rutuku, Sangema, Victoria , whereas Mbarara had only two varieties; Victoria and Musitamya. Varieties *Maboni* and *Wafurika* where found only in Mbale in addition to Sangema and Victoria.

3.3.2 Cropping systems of surveyed districts and their influence on late blight incidence and severity

The districts surveyed had different cropping systems. In Kabale and Mbale, potatoes were mostly grown as a monocrop. In Mbale, the farming system in the area was predominantly a coffee-banana system, but on a few occasions, potato was intercropped with bananas or coffee. In Mbarara, potatoes were found commonly intercropped with beans (*Phaseolus vulgaris* L) or field peas (*Pisum sativa*) and on a few occasions with maize (*Zea mays* L). In Kisoro, potatoes were mixed cropped with *Phaseolus* beans, maize and sorghum (*Sorghum bicolor*), and occasionally, grown in pure stands.

In all the districts surveyed, except Mbarara, pure stands potatoes (monocrops) recorded the highest amount of late blight with mean severity of more than 20% (Table 1). In Kisoro, potato+bean intercrop recorded severity of 20%, and the potato+maize or potato+sorghum registered 18.7%. In Mbarara, potato+maize intercrop recorded the highest severity (10.2%). In case of potato+banana intercrop recorded 23.6% severity and the least was from the potato+coffee system (10.9%).

Table 1 Influence of cropping system on incidence and severity of late blight in Uganda, 1997-1999.

Cropping system	Percent incidence	Percent severity
<u>Kisoro district</u>		
Potato+beans	71.0	20.0
Potato+maize or potato+shorghum	70.0	18.7
Pure stand (monocrop)	75.0	20.4
<u>Kabale district</u>		
Pure stand	85.9	28.3
<u>Mbarara district</u>		
Potato+beans or peas+potato	44.9	8.2
Potato+maize	42.0	10.2
Pure stand	30.1	7.9
<u>Mbale district</u>		
Potato+banana	49.2	23.6
Potato+coffee	42.0	10.9
Potato+peas	46.0	19.2
Pure stand	55.4	29.5

Pooled mean incidence and severities of different potato varieties during the four seasons

3.3.3 Late blight occurrence in surveyed districts

Late blight incidence and severity varied significantly ($P \leq 0.05$) from district to district and season to season during the four surveys. Additionally, location (district) x season interaction was highly significant (Table 2). During the first survey in December 1997, Mbale district recorded the highest amount of disease (99.1% incidence with 58.8% severity), Kabale recorded the least (82.6% incidence and 22.9% severity (Table 3).

During the second survey in May 1998, disease severity in all the surveyed districts was lower than 20 % with Kabale recording the highest (16.6%). In November 1998 and May 1999, Kabale district still had the highest amount of disease with severities of 39% and 31.8%, respectively.

Overall, Kabale district consistently recorded the highest late blight severities, with mean severity of 27.6%, followed by Mbale (21.8%); Mbarara consistently recorded the least, with mean severity of only 10.8%. Considering all the 4 seasons, there was more disease during December 1997, with mean severity of 40.2%, while the least severity was in May 1998.

3.3.4 Susceptibility of different potato varieties to late blight

The highest severity was recorded on Victoria (62.1%) followed by Wafrika (61.8%). Sangema (59.5%) and Maboni (48.2%). In all the districts, Victoria, Sangema and Kabale had consistently higher late blight severities, while Musitamya and Nakpot 1 (382171.4) had the least (Table 4).

During the survey, it was noted that potato farmers used fungicides to control late blight. Dithane M-45 (Mancozeb 80% WP) was the most common fungicide in all the four districts. Occasionally, Ridomil (systemic fungicide) was used in Kisoro, but more commonly in Kabale district. Where Ridomil was used, farmers applied it in

Table 2 Mean squares of transformed¹ percentage incidence and severity of potato late blight in Uganda, 1997-1999.

Source	DF	Mean squares	
		% incidence	% severity
Fields	37	395.9 ns ²	213.1 ns
Seasons	3	8936.0**	3895.5**
Districts	3	15487.3**	1374.4**
Seasons x Districts	8	8545.9**	2888.4**
Error	262	514.2	151.4

¹ Arcsine transformation ($x+1$); ² ns denotes not significant and ** significant at $P \leq 0.001$ probability level.

Table 3 Incidence(%) and severity(%) of potato late blight in Uganda, 1997-1999.

District	Dec. 1997		May 1998		Nov. 1998		May 1999		Mean ³	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Kisoro	88.3 abc	38.7 b	73.9 cd	12.6 cde	85.4 bc	20.4 c	52.1 e	7.1 def	74.9	19.7
Kabale	82.6 cd	22.9 c	90.2 abc	16.6 cd	95.2 ab	39.0 b	81.6 bc	31.8 b	87.4	27.6
Mbarara	-	-	65.9 de	10.0 def	23.6 f	9.7 def	27.9 f	6.4 ef	39.1	8.7
Mbale	99.3 a	58.8 a	25.3 f	4.1 cf	5.0 f	4.5 f	66.9 de	19.7 c	49.1	21.8
Mean ³	90.1	40.2	63.8	10.8	52.3	18.4	57.1	16.3	62.6	19.5

¹ Means in each column and row with similar letter(s) do not differ significantly at ($P \leq 0.05$) using t mean Probability difference (PD); Mean separations were based on transformed % incidence and severity using Arcsine transformation ($x+1$), but values presented are the untransformed values

² Late blight rating scale 0-100% (Henfling, 1987);

³ Means pooled across locations and seasons in last column and row, respectively.

combination with Dithane M-45 (contact fungicide). In addition to the two fungicides farmers in Kisoro and Mbale used copper compounds. The frequencies of fungicide use was higher in Mbale district, with a mean of five sprays per season (Table 5). In Kisoro and Kabale farmers applied on average 3 sprays per crop, but those in Mbarara district applied only one spray per crop.

Table 4 Mean late blight severities (%) on potato varieties in selected districts of Uganda during 1997- 1999

Varieties	Dec. 1997	May 1998	Nov. 1998	May 1999
<u>Kisoro district</u>				
Cruza	42.7	12.4	16.1	4.1
Kabale	20.0	-	41.9	-
Mbumbamagara	-	-	-	20.4
Musitamya	-	-	11.6	-
Rutuku	38.4	10.4	18.4	5.7
Sangema	45.0	-	-	5.0
Victoria	46.2	5.8	24.7	4.5
<u>Kabale district</u>				
Cruza	-	-	40.7	18.9
Kabale	26.2	18.6	65.7	-
Kisoro	-	-	49.5	58.3
Malirahinda	-	6.6	42.2	6.3
Mbumbamagara	-	12.1	22.5	50.0
Musitamya	-	-	-	0.0
Rutuku	-	15.4	32.1	46.1
Sangema	31.7	15.7	53.5	14.6
Victoria	12.8	14.8	42.4	36.6
Nakpot 1 (382171.4)	-	-	-	0.0
<u>Mbarara district</u>				
Musitamya	*	-	0.1	0.3
Victoria	*	5.7	10.9	8.2
<u>Mbale district</u>				
Maboni	48.2	0.5	0.3	16.3
Sangema	59.5	27.0	-	-
Victoria	62.1	0.5	-	-
Wafurika	61.8	8.8	-	20.5

*Survey not conducted during the season; Late blight rating scale 0-100% (Henfling, 1987)

Table 5 Frequency of fungicides application in the surveyed districts of Uganda

District	Type of fungicides	Number of sprays per season
Kisoro	Dithane M-45, Ridomil*, Copper compounds	2
Kabale	Dithane M-45, Ridomil*	3
Mbarara	Dithane M-45	1**
Mbale	Dithane M-45 , Copper compounds	5

* Used in combination with Dithane M-45; ** In most cases no fungicide is applied.

3.3.5 Relationship between potato late blight and tuber yield

The relationship between potato late blight and tuber yield was assessed only in Kabale and Mbarara districts during the second season of 1998. Late blight severities and tuber yield varied significantly ($P \leq 0.05$) within fields sampled, and also between the two districts. Additionally, location (district) x potato field interaction was significant (Table 6). Disease levels varied from variety to variety, with Victoria registering the highest amount of disease (48.3% severity), and Musitamya the least (5% severity). Other varieties had mean severities ranging between 5 to about 38.3% (Table 7). Likewise, average tuber yield varied from variety to variety with Sangema recording the highest (16.9 t/ha) and Musitamya the least (8.2 t/ha). Tuber yields averaged 14.6 and 7.1 t/ha for Kabale and Mbarara plots, respectively. Considering tuber damage, most varieties had healthy tubers (about 70%) and less than 10% were affected by late blight (Table 8). The most prevalent pests that attacked potato tubers were millipedes and nematodes.

There was only a weak negative correlation between late blight severity and yield of potatoes in the two districts ($r=-0.065$, $P=0.71$).

3.3.6 Management of potato fields

Potato were planted at an average spacing of 75 cm x 30 cm and in pure stands in the fields studied. The fields were planted during the second week of October 1998, and were weeded only once. The majority of farmers commonly used Dithane M-45 to control late blight. Farmers in Kabale sprayed on average 3 times, while in Mbarara most farmers only used one spray during the season. In case of Kabale, potatoes were grown mostly on the lower slopes (66.7%) and valley bottoms (33.3%). For Mbarara potatoes were mostly grown on flat hill tops (83.3%) and slopes (16.7%). In Kabale black soil (erikwiragura) or lumb (enombe) were the common soil type used for potato cultivation. In Mbarara, the soils were mostly light (sandy loams). None of the farmers sampled had applied organic or inorganic fertilizers to their fields.

Table 6 Mean squares of late blight severities (%) and potato tuber yield (t/ha) in Kabale and Mbarara during the second season of 1998.

Source	DF	Percent severity	Tuber yield (t/ha)
Sampling positions	2	243.8*	14.1 ns
Potato fields (PF)	5	1688.0**	31.8**
Districts	1	1406.3**	504.0**
District x PF	5	2387.9**	18.7*
Error	22	36.2	5.6

ns denotes not significant, * & ** significant at $P \leq 0.05$ and $P \leq 0.001$ probability level, respectively.

Table 7 Late blight severities and tuber yield of different potato varieties in Kabale and Mbarara

Varieties	No of fields	Percent severity				Yield
		1st visit	2nd visit	3rd visit	Mean	T/ha
Cruza	1	10.0	30.0	40.0	26.6 b	12.8 bc
Musitamya	1	0.0	5.0	10.0	5.0 d	8.2 d
Rutuku	1	10.0	15.0	20.0	15.0 c	15.1 ab
Sangema	1	25.0	50.0	40.0	38.3 a	16.9 a
Victoria	8	35.0	50.0	60.0	48.3 a	9.7 cd

1st visit=vegetative growth; 2nd visit= flowering stage; 3rd visit=Physiological maturity
For mean late blight severities and tuber yields, means with the same letters are not significantly different ($P \leq 0.05$) using ls mean probability difference.

Table 8 Relative tuber damage by pests and diseases from a sample of 60 tubers per plot

Variety	Healthy		Late blight		Millipedes		Nematodes	
	No	%	No	%	No	%	No	%
Cruza	50	83.3	2	3.3	6	10.0	4	6.7
Musitamya	46	76.7	1	1.7	10	16.7	4	6.7
Rutuku	47	78.3	4	6.7	9	15.0	1	1.7
Sangema	45	75.0	3	5.0	6	10.0	1	1.7
Victoria	45	75.0	5	8.3	11	18.3	2	3.3

Mean data for 12 potato fields sampled in Kabale and Mbarara

3.4 Discussion

Variation in late blight incidence and severity among the four districts was probably related to weather conditions (Apendix 1). Except for Mbale district which had the greatest amount of disease in December 1997, there was generally more late blight in Kabale district. In 1997, Mbale district recorded rainfall above 200 mm per month just before the survey, which must have induced high late blight attack.

Varieties Victoria, Sangema and Kabale consistently recorded higher late blight severities in all the districts and seasons. These varieties are thought to possess the R-genes (Umaerus *et al.*, 1983), which may have succumbed to *P. infestans* pathotypes over the years. This calls for continued effort to develop potato varieties with high levels of horizontal resistance which would resist the attack of new virulent populations of *P. infestans* (Deah *et al.*, 1995; Goodwin *et al.*, 1996). In Uganda, there are on-going efforts to develop potato varieties with horizontal (durable) resistance. Some promising clones with higher levels of horizontal resistance and yield have been identified in population B genotypes under evaluation at Kalengyere Research Station (Hakiza *et al.*, 1999).

The higher levels of late blight on the common varieties may also partially be due to the failure of the available fungicides to control late blight, and improper use of fungicides (poor timing, low dosage, etc). Lack of late blight control is also suspected to be due to the appearance of metalaxyl resistant strains of *P. infestans* (Deah *et al.*, 1995; Goodwin *et al.*, 1996). A number of farmers used fungicides to control late blight on potatoes, Mbale farmers recording the highest frequency of application. Although there was more use of fungicides in Mbale than other districts, late blight was still severe in Mbale. In Kabale and Kisoro districts, there was evidence of use of the systemic fungicide ridomil. Studies elsewhere eg. in Netherlands and Ireland (Davidse *et al.*, 1981; Dowley and O'Sullivan, 1981) have reported the development of *P. infestans* phenotypes resistant to metalaxyl, which were detected after the use of phenylamide compounds to which metalaxyl, an ingredient of ridomil belongs. There is therefore a possibility that resistant phenotypes may have developed in Uganda and have contributed to the increased occurrence of late blight. In order to understand the possible occurrence of

fungicide resistant *P. infestans* strains in Uganda, a metalaxyl resistance study was conducted and is reported in chapter four.

Victoria was the commonly grown variety in the four districts studied. This variety has wide adaptability (Sikka, 1994) and a high market value, because of its pinkish skin colour. Kabale district had the highest number of varieties encountered during the survey, likely because farmers in this district have access to the released varieties from Kalengyere Research Station. Also, conditions in Kabale district are very favourable for potato production (Sikka *et al.*, 1991). Two varieties *Maboni* and *Wafurika* were found only in Mbale district. These varieties are believed to have been obtained from across the boarder in Kenya, because they do not appear anywhere as released varieties by the Uganda National Potato Program.

Potatoes grown as pure stands (monocrops) recorded the highest amount of disease in all the districts. The high disease severity could be associated with the fact that, when potatoes are grown alone, they tend to form a canopy on establishment, thus creating a conducive microclimate for late blight development. In Kisoro, potato+bean intercrop also recorded high late blight severity. In this district, farmers commonly grow climbing beans, such that when grown in an intercrop with potatoes, this probably creates conducive environment for late blight development. Contrastingly, there was less late blight when potato was intercropped with the bush type of beans as was the case in Mbale and Mbarara.

There was considerable variation in both disease severity and tuber yield among the potato fields sampled in Kabale and Mbarara. Disease levels also varied, from variety to variety, Victoria and Sangema recording the highest severities. Contrastingly, Cruza, Musitamya and Rutuku registered relatively low late blight severities (Table 7). These later varieties probably possess high resistance to late blight. Interestingly, Sangema registered the highest yield of 16.9 t/ha despite its high susceptibility to late blight (38.3%), a possible indication of its tolerance to late blight. It was also noted that some farmers had stayed with the same potato seed stocks for over 6 years, which likely contributed to the low yields.

There was low tuber infection by late blight in all the varieties studied, which might be associated with the inherent resistance of the varieties to tuber infection (Stewart *et al.*, 1994). Furthermore, dry conditions prevailed during the study, which probably led to the low tuber infection. The study also documented that millipeds are a serious potato tuber pest. They affect the quality and market value of the tubers. Research should address this problem, since no work been done on it in the country.

Overall, there was weak association between late blight severity and yield of potatoes. There were two main reasons for this. First, most of the farmers had applied fungicides to control late blight, hence the low disease levels. Also late blight yield loss study was only conducted during October-December 1998, a season which was exceptionally dry and therefore, only mild late blight developed.

The average yields of 14.6 and 7.1 t/ha for Kabale and Mbarara, respectively, are quite low compared to on-station yields (26 t/ha) at Kalengyere. The low yields might be related to low soil fertility. None of the farmers interviewed had applied organic or inorganic fertilizer in the fields sampled. The relatively high yields in Kabale is probably related to the more fertile volcanic soils in the district, and accessibility to high quality seed from Kalengyere Research Station. Also, the aphid population in the highlands areas of Kabale is low (Kakuhenzire, 1996), thus leading to decreased rate of potato seed degeneration from aphid transmitted viruses.

CHAPTER FOUR

POPULATION STRUCTURE OF *Phytophthora infestans* IN UGANDA

4.1 Introduction

Phytophthora infestans has its centre of origin in Mexico where mating types A1 and A2 exist, and both mating types must normally be present for the formation of oospores (Tooley *et al.*, 1985). It was the A1 mating type that spread worldwide. Information on population dynamics of *P. infestans* indicates that A2 mating type belongs to a new sexual population that carries both A1 and A2 mating types (Fry *et al.*, 1992). The new populations are rapidly replacing the old ones in many regions (Fry *et al.*, 1993), and they carry a potential to complete their sexual cycle. They also tend to have higher levels of virulence and high frequencies of resistance against metalaxyl (Deahl *et al.*, 1995; Goodwin *et al.*, 1996).

Considering the possible existence of the new population of *P. infestans* in eastern Africa, strategies to reduce their potential destructive effects to potato production include; breeding resistant potato varieties and integrated disease management. To do this it would be helpful to acquire more knowledge on the population structure of the fungus in the region. The study was conducted to characterise *P. infestans* populations in Uganda with respect to possible occurrence of A2 mating type, pathogen resistance to metalaxyl, and virulence of the fungus on potato and tomato hosts.

4.2 Materials and methods

4.2.1 Study area and sample collection

Samples of potato and tomato infected with late blight were collected from 46 sites in the districts of Kisoro, Kabale, Mbarara and Mbale. This was done in May 1998. At all sites samples were collected by randomly picking infected leaves from each field, measuring approximately 0.5 to 1.0 hectares. Sampling was done every 15 to 20 km of a road system across each district. In Kisoro district samples were collected from Kashenyi, Mukateretere-Kanaba, Nyakabande, Nyarusiza, Maziba and Busanza. In Kabale samples were collected from Muko, Ikumba, Bufundi, Hamurwa and Bubare in Rubanda county; Rwamucucu, Kashambya and Bukinda in Rukiga county; and Kamuganguzi, Kitumba and Katuna valley in Ndorwa county. In Mbale the samples were from Mt. Elgon area, and in Mbarara from the highlands of Mwizi-Bugamba in Rwampara and Isingiro, these being the key potato growing areas.

Thirteen samples were collected from Kisoro district, 16 Kabale, 7 Mbarara and 10 from Mbale. About 10 leaflets were collected per field sample, purposely from younger lesions with sufficient green tissue for the lesion to develop (Plate 1). Samples were put in plastic bags, sealed (not air tight), labelled and transported in a cool box (approximately 15°C) to Namulonge Agricultural Research Institute laboratory for isolation of *P. infestans*. The following information was also recorded; geographical location, altitude, host cultivar, plot size, fungicide used, number of sprays and disease severity.



Plate 1. Young expanding lesions of late blight on a potato plant

For the purposes of recovering sufficient number of isolates, two more collections were done in November 1998 and May 1999 from 48 and 42 sites, respectively. Sampling was done in the same locations, although not necessarily from the same sites. A total of 136 late blight samples were collected.

4.2.2 Laboratory isolation of *P. infestans*

Sample bags were opened within two days of collection and were spread on clean paper towels. The best lesions (young, spreading, on well preserved host tissue) were selected. The leaves were then rinsed in distilled water, and placed into water agar plates to maintain high humidity. The plates were then incubated overnight at 17 to 20°C and checked for sporulation the following day.

Infected leaf pieces (approximately 5mm x 5mm) were cut from lesion borders, so that half the piece was green, i.e., healthy, and the other brown, i.e., infected. One piece was taken from sporulating lesion and following successful isolation, was considered *P. infestans* isolate. Four infected leaves were selected from each field sample.

Leaf pieces were first surface disinfected by immersing in 5% bleach (NaOCl) for about 1 minute and then rinsed 3 times in sterile distilled water. The infected leaf piece was then placed under a thin, surface disinfected slice of potato tuber (approximately 8mm x 3mm x 5mm) of variety Victoria (CIP381381.20) in inverted water agar plates (prepared by autocaving 15g of agar in 1 litre of distilled water for 15 min at 15 psi and

poured on plates after cooling). They were incubated at 17°C to 20 °C for seven 7 days to allow for mycelial growth through the potato slice.

Mycelia from the surface of the slice were picked and aseptically transferred to selective (SEL) V8 medium (Hohl, 1991) to avoid bacterial contamination. Selective (SEL) V8 medium was prepared by adding antibiotics/fungicides mix which included the following in mg/L; grieseofulvin (20), nystatin (19), benlate (10), methoxypurine (5), rifamycin (30), nalidixic acid (5), 8-azaguanine (40), neomycin (30) (Hohl, 1991) to 10 % V8 medium at approximately 40°C. The 10% V8 medium was prepared by combining V8-juice (100 ml), distilled water (1 L) , CaCO₃ (1.0 g), vegetable oil (1 ml) to promote sporulation of *P.infestans* (Klemmer and Lenney, 1965), bacteriological agar (15g) and autoclaving at 15 psi for 20 min. The plates were incubated at 17 to 20°C, and after seven days of incubation sporangia were picked from culture plates and transferred onto 10% V8 medium for subsequent studies. Twenty five isolates were recovered from the May 1998 collections, 26 from November 1998 and 30 from the May 1999 collections. Thus, a total of 81 isolates were recovered.

4.2.3 Metalaxyl resistance test

Sensitivity to metalaxyl was determined for the 81 isolates using 10% V8 medium supplemented with 90.6% technical grade metalaxyl (Ciba, Switzerland), at concentrations of 0, 5 and 100 µg/ml metalaxyl, replicated 4 times. Uniform sized agar plugs approximately 5 mm in size were cut from the edge of actively growing *P. infestans* cultures (10-14 days old), using a surface sterile blade, putting one plug at the centre per

plate. After 21 days of incubation at 17-20°C, radial growth was measured. For each seasons collection, the procedure was repeated thrice and data collected subjected to Analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS package(SAS, 1987). Although the ANOVA indicated significant differences ($P \leq 0.05$) among the repeat tests (Appendix 2), the data were pooled for ease of data presentation, and the trends were generally consistent.

Estimates of the growth rate relative to the control (0 $\mu\text{g/ml}$ metalaxyl) were also calculated. Subsequently, isolates were separated into groups depending on their sensitivity to metalaxyl following the procedures used at CIP (Forbes, 1997). Sensitive isolates were those with growth $< 40\%$ of the control at 5 and 100 $\mu\text{g/ml}$; intermediate isolates had a growth $\geq 40\%$ of the control at 5 $\mu\text{g/ml}$ but less than 40% of the control at 100 $\mu\text{g/ml}$; resistant isolates had growth $\geq 40\%$ of the control at 100 $\mu\text{g/ml}$. The numbers in each of the metalaxyl reaction groups were analysed by; a) season where districts were considered replications, and b) location where seasons were considered replications. Means were separated by Least Squares procedure of the SAS package (SAS, 1987)

4.2.4 Mating type test

Mating type was determined on 80 of 81 isolates (1 isolate was lost due to contamination) by placing a plug of an unknown isolate on one side of a petri dish containing 10% clarified V8 medium (prepared by centrifuging 150 ml of V8-juice for 5 min at maximum speed (6500 revolutions per minute) then 100 ml of the supernatant used for medium as

described earlier with a plug of a known isolate A1 (1724) obtained from Kenya. Each isolate was replicated in 4 plates. The same set of isolates were also grown in pure cultures (selfing). The plates were incubated at 17-20° C for 21 days after which formation of oospores were observed under a binocular microscope at low magnification (40 X) which covers an area of about 35% of the plate. Counting of oospores was done by turning the plates upside down and focusing on the bottom of the plate. For mating, oospore search was done along the overlap area of the two cultures and also along the edges of the plates. For selfing, oospore search was also along the edges of the plate, where most oospores are formed especially if only few oospores are formed (H. Hohl, *Personal commun*). The transformed data on oospore count (using square root transformation $x+0.5$) were subjected to Analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS statistical package (SAS, 1987). Each experiment repeat was analysed separately, but data were also pooled means and analysed. The number of isolates in various oospore production categories ie., non oospore producers, oospores producers in selfing only, oospore producers in mating only, and oospore producers in both mating and selfing, were analysed by; a) season where districts were considered replications, and b) location where seasons were considered replications. Means were compared using the Least Squares means procedure of the SAS package (SAS, 1987)

4.2.5 Virulence of *P. infestans* isolates

Virulence of the isolates was tested on potato variety Victoria (CIP 381381.20) and tomato variety Maglobe, both of which have high level of susceptibility to late blight (*Personal observation*). The tomato and potato plants used in these tests were raised in the screenhouse where day time temperatures averaged $22 \pm 4^{\circ}\text{C}$. Potato leaves were detached from plants which were 6 - 8 weeks old, and the younger, upper leaflets were used. Tomato leaves were from 8 weeks-old plants. Leaves were collected immediately before use, washed several times with sterile distilled water for about 20 minutes. The leaves were later blotted slightly on paper towels to remove excess moisture. Bases of the leaflets were covered with pieces of moist cotton wool to reduce leaf desiccation. The leaves were then placed upside down (abaxial side up) into 9 cm plastic petridishes containing moistened filter paper. Two leaflets were placed in each petridish.

Inoculation of the leaflets was done following the method described in the "Manual for Laboratory Work on *Phytophthora infestans* at CIP, Qito" (Forbes, 1997). Sporangial suspensions were produced from cultures according to the procedures earlier described. Sporangial concentrations were determined with a haemocytometer and adjusted to about 60,000 sporangia per millilitre. The sporangia were incubated in a refrigerator at 4°C for 2 hours to induce zoospore germination. Three drops of $10\mu\text{l}$ of sporangial suspension (containing about 700 sporangia) were then applied to the two leaflets, mostly over the mid rib. The inoculations were replicated in four plates, and were later incubated at $17-20^{\circ}\text{C}$ for seven days. Subsequently, the leaflets were assessed for percentage leaf area

blighted and lesion length. The experiment was repeated three times for each seasons collection of May 1998, November 1998 and May 1999.

The data collected were transformed (using square root transformation ($x+0.5$) for lesion length, and arcsine transformation ($x+1$) for percent area blighted) before analysis of variance (ANOVA) using general linear model (GLM) procedure of the SAS statistical package (SAS, 1987). Means of the transformed data were separated by the Least Squares. For each season, the data were also pooled, even though the ANOVA indicated significant differences ($P \leq 0.05$) among the repeat tests (Appendix 3). This was done for ease of data presentation, and the general trend was consistent across the repeats. An isolate was considered virulent on the host leaves when the mean sporulating lesions were ≥ 1 cm in length.

4.3 Results

4.3.1 Isolation of *P. infestans*

A total of 39 isolates of *P. infestans* were recovered from samples collected from Kabale district, 20 from Mbarara, 14 from Mbale and only 8 from Kisoro (Table 9). For several samples yellowish bacterial slime developed on the potato slices in about 5 to 6 days, rendering futile any attempt to isolate *P. infestans*. Such samples were infected with *Ralstonia solanacearum*. This reduced the number of *P. infestans* isolates recovered from the samples, especially those from Kisoro and Mbale (Table 9). None of the 10 tomato samples collected yielded *P. infestans* with the potato slice method. Some attempt

to grow them through the tomato fruit yielded two isolates, which were difficult to maintain or grow well on the 10% V8 medium available in Uganda. Thus, further studies concentrated on the potato *P. infestans* isolates.

Table 9 Number of *P. infestans* isolates recovered from potato leaves in 1998-1999

District	May 1998	November 1998	May 1999	Total
Kisoro	3 (13)	3 (11)	2 (11)	8 (35)
Kabale	3 (16)	15(15)	21(12)	39(43)
Mbarara	13(7)	5 (9)	2 (9)	20(25)
Mbale	6 (10)	3 (13)	5 (10)	14(33)
Total	25(46)	26(48)	30(42)	81(136)

Numbers in parenthesis indicate number of samples collected

4.3.2 Metalaxyl resistance test

Differences in radial growth rates for the 3 metalaxyl concentration were highly significant ($P < 0.05$) among the isolates tested, and addition of metalaxyl to culture medium significantly ($P < 0.05$) reduced growth rates in most of the isolates tested (Table 10, Plate 2). Among the May 1998 collection, mycelial growth of the isolates was better on metalaxyl-free media with growth rates ranging between 55 mm to 73.1 mm for isolates 3010 and 2002, respectively. Based on the predetermined classification of the isolates as resistant, intermediate and sensitive to metalaxyl (Forbes, 1997), 11 were grouped as resistant (e.g 1002), 7 as intermediate (e.g 3003), and 7 as sensitive (eg. 1001) (Table 11). The resistant *P. infestans* isolates had their mycelial growth on media with metalaxyl ranging between 21.7 mm for isolate 4002 to 56.0 mm in diameter for isolate 2002. For the intermediate isolates, their mycelial

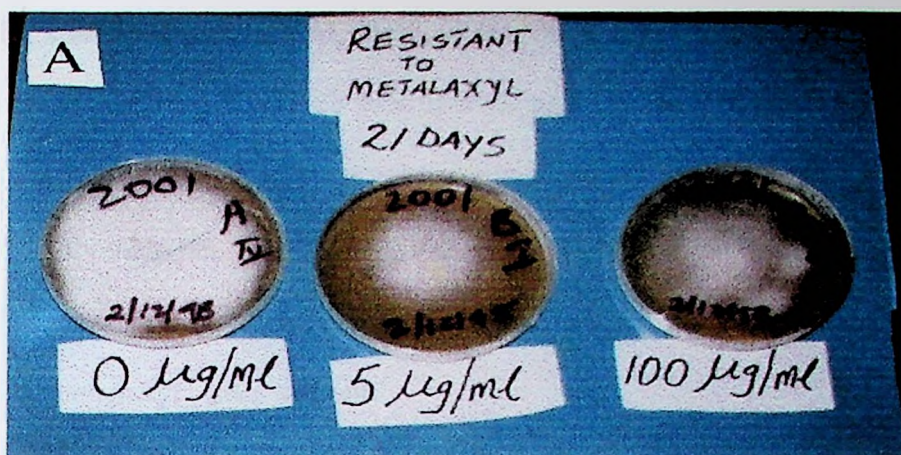


Plate 2. Resistant (A), intermediate (B) and sensitive (C) reactions shown by Ugandan isolates of *P. infestans* to metalaxyl

growth ranged from 28.6 mm to 35.8 for isolates 3005 and 4005, respectively. Sensitive isolates, for example, 1001, 1003, 2003, 3002, 3007, 3011, 4004, and the tester (A1) 1724 had their mycelial growth of less than 20 mm in diameter when they were grown on media with metalaxyl. Interestingly, some of the *P. infestans* isolates collected in May 1998, for example, 2002, 3010, 4001 grew better on medium amended with 100 µg/ml of metalaxyl than on 5 µg/ml. Surprising still, isolate 2002 grew even better at 100 µg/ml of metalaxyl than without.

Among the November 1998 isolates, for the metalaxyl resistant isolates, isolate 3020 had the biggest colony diameter of 48.2 mm while 4009 had 31.1 mm on media with 100 µg/ml. The range for the intermediate was 24.2 mm for isolate 4007 to 42.1 mm for isolate 2005, on media with 5 µg/ml (Table 11). Sensitive isolates had growth rates of less than 20 mm in diameter on media with metalaxyl. All the November 1998 isolates grew better on media without metalaxyl, and their growth ranged between 59.0 mm to 72.7 mm in diameter for isolates 2015 and 1004, respectively. However, isolates 2021, 3017, 4008 grew better on media with 100 µg/ml better than on media with 5 µg/ml.

Growth rates for the May 1999 *P. infestans* collection on media with metalaxyl ranged from 21.7 mm for isolate 2028 to 50.0 mm for isolates 2036 and 2046 on media with 100 µg/ml (Table 11). For the intermediates, growth rates ranged between 22.1 mm for isolate 2025 to 37.6 mm for isolate 4014 on media with metalaxyl. Sensitive ones had their colony diameters ranging from 9.5 to 21.5 mm. As with the November collection, radial growth rates were higher on media without metalaxyl. Isolates 2036, 2046, 4012 had better growth on media with 100 µg/ml than on 5 µg/ml. Results of radial growth

of repeat tests were significantly different ($P \leq 0.05$), indicating that the isolates were highly variable under different metalaxyl concentration tested.

The number of *P. infestans* isolates that were highly resistant, intermediate and sensitive to metalaxyl from different parts of Uganda did not vary significantly ($P \geq 0.05$) with season the isolates were collected. For the May 1998 collections, the majority were highly resistant, and there were equal number of intermediates and sensitive isolates (Table 12). In the case of the November 1998 collection, the majority were sensitive, while for May 1999 collection, an overwhelming majority were resistant (Table 12). Overall 44.4% (36 of 81) of the isolates tested were highly resistant to metalaxyl, 23.5% showed intermediate resistance and 31.2% as sensitive (Table 12).

Interestingly, the number of isolates that were grouped as resistant, intermediate and sensitive varied significantly with location the isolates were collected (Table 13). Fifty percent (18 of 36) of highly resistant *P. infestans* isolates were from Kabale district with a mean of 6.0 resistant isolates per season, while Kisoro registered only 2.8% with mean of 0.3. Mbarara and Mbale had 25 and 22.2% of the highly resistant isolates, respectively with means of 3.0 and 2.7 per season, respectively (Table 14). Kisoro isolates compromised 15.8 % (3 of 19) of the intermediate isolates, Kabale and Mbale each (26.3%) and Mbarara 31.6 % (Table 14). Kabale district had the majority of the sensitive isolates (61.5%) while Mbale had only 3.9% sensitive ones.

Table 10 Mean squares of colony diameter (mm) of *P. infestans* isolates under different metalaxyl concentrations for May and November 1998, and May 1999 collection

Source	DF	1st run	2nd run	3rd run	Mean
May 1998 collection					
Replication	3	21.2 ns	20.5 ns	32.5*	10.1*
Isolates	25	858.3**	1049.6**	1167.3**	941.3**
Metalaxyl concentration (MC)	2	51023.3**	67050.4**	51955.8**	56415.3**
Isolates x MC	50	173.8**	221.5**	244.9**	158.2**
Error	231	9.4	9.5	12.5	3.7
November 1998 collection					
Replication	3	21.5 ns	3.9 ns	58.4*	7.6 ns
Isolates	25	820.4**	926.6**	1037.2**	868.7**
Metalaxyl concentration (MC)	2	75992.2**	61422.1**	62384.5**	66400.5**
Isolates x MC	50	185.9**	214.8**	263.3**	189.7**
Error	231	8.4	9.9	17.5	5.3
May 1999 collection					
Replication	3	41.7*	13.6 ns	9.2 ns	2.1ns
Isolates	29	1073.6**	864.2**	931.5**	913.4**
Metalaxyl concentration (MC)	2	43812.1**	49172.6**	50570.7**	47792.3**
Isolates x MC	58	275.0**	256.2**	188.9**	217.4**
Error	267	13.1	13.3	15.8	6.2

ns, * and ** denote not significant, and significant at $P \leq 0.05$ and ≤ 0.001 , respectively

Table 11 Mean colony diameter (mm) of *P. infestans* isolates at different metaxyl concentrations after 21 days of incubation for the May 1998, November 1998 and May 1999 collection

Colony diameter (mm)											
May 1998				November 1998				May 1999			
Isolates	0µg/ml	5µg/ml	100µg/ml	Isolates	0µg/ml	5µg/ml	100µg/ml	Isolates	0µg/ml	5µg/ml	100µg/ml
1001	61.5 ij	20.3 i	17.2 g	1004*	72.7 a	26.6 fg	12.4 hij	1008	63.6 efghi	14.4	12.7
1002**	66.8 efg	29.0 g	23.6 g	1005	61.3 jkl	15.6 hijk	10.6 j	1009*	65.4 cdef	32.2 ij	21.0 ghi
1003	63.8 hi	14.1 j	12.4 ij	1007*	61.0 jkl	24.4 g	19.7 f	2024	71.7 a	21.5 m	22.2 g
2001**	69.7 bc	44.8 b	49.4 b	2004	66.6 efgh	16.4 hi	10.9 j	2025*	60.5 hijk	22.1 lm	16.5 j
2002**	73.1 a	66.7 a	56.0 a	2005*	69.0 bcdef	42.1 c	24.1 e	2026**	60.3 hijkl	30.4 jk	29.6 f
2003	65.3 g	14.3 j	8.7 k	2006	70.0 abcd	15.0 jk	10.4 j	2027	60.0 ijkl	13.5	10.6
2008	66.5 efgh	24.3 g	12.4 hij	2008	66.5 efgh	24.3 g	12.4 hij	2028**	54.6 m	22.7 l	21.7 gh
3001**	68.8 bcd	28.8 g	21.9 g	2009**	71.5 ab	43.9 c	34.8 c	2029	68.3 abc	15.8	12.5
3002	60.6 k	18.7 i	11.6 ij	2010	66.2 fghi	13.1 k	11.5 ij	2030	60.9 ghij	15.8	12.9
3003*	66.8 efg	31.8 ef	10.6 j	2013	63.8 hij	14.4 jk	10.9 j	2031**	58.9 jkl	31.2 j	29.6 f
3004*	65.9 l	25.6 h	9.5 k	2014	65.6 ghi	15.2 jk	15.2 gh	2032*	57.1 klm	35.4 ghi	10.3
3005*	65.7 g	28.6g	12.8 ij	2015	59.2 l	18.7 h	11.7 ij	2033**	66.6 cdef	33.2 hi	37.1 d
3006*	63.9 hi	30.5 fg	16.6 h	2016*	69.9 abcd	31.2 e	24.9 e	2034**	66.0 cdef	38.5 efg	30.4 f
3007	65.0 gh	18.5 i	11.0 j	2017*	64.1 hij	28.6 fg	17.3 fg	2035**	67.3 cd	51.4 ab	47.3 ab
3008**	68.5 cdef	35.8 d	32.0 e	2018	63.3 ijk	4.0 jk	11.4 ij	2036**	67.0 cde	54.7 a	50.4 a
3009**	71.3 ab	36.5 d	34.7 d	2021**	70.0 abcd	38.7 d	34.0 cd	2037**	60.3 hijkl	38.9 ef	34.1 de
3010**	55.0 l	28.0 gh	31.8 f	2022**	60.6 kl	25.7 g	19.5 f	2038**	66.3 cdef	49.6 bc	43.9 bc
3011	61.0 jk	18.4 i	15.6 h	2023	66.9 defgh	18.1 hi	10.3 j	2039**	71.5 a	47.1 c	31.9 ef
3012**	67.0 efg	30.0 fg	23.5 g	3014	70.0 abcd	13.6 jk	10.9 ij	2040**	67.5 bcd	41.7 de	40.9 c
3013*	69.1 bcde	29.4 fg	21.9 g	3015**	67.5 cdefg	56.0 b	38.0 b	2042**	64.7 def	31.8 j	31.2 ef
4001**	70.0 bc	34.1 de	36.0 d	3016*	69.1 bcdef	26.3 fg	12.2 hij	2043	59.5 jkl	13.3	9.5
4002**	67.0 defg	29.4 fg	21.7 g	3017**	69.5 bcde	25.4 g	34.7 c	2045	66.9 cde	16.1	15.0
4003*	69.6 bcd	30.3 fg	22.0 g	3020**	70.9 ab	62.4 a	48.2 a	2046**	70.9 ab	50.3 bc	50.3 a
4004	61.9 i	18.2 i	13.8 h	4007*	66.1 fghi	24.2 g	13.3 hi	3021**	63.7 efgh	36.4 fgh	36.4 d
4005*	66.1 fg	35.8 d	20.9 g	4008**	70.2 abc	29.2 ef	32.1 cd	3022	64.1 defg	22.1 lm	17.5 j

Table 11 continued

Colony diameter (mm)																					
May 1998				November 1998				May 1999													
Isolates				Isolates				Isolates													
0µg/ml				0µg/ml				0µg/ml													
5µg/ml				5µg/ml				5µg/ml													
100µg/ml				100µg/ml				100µg/ml													
4006**	69.4	bcd	e	40.4	c	39.4	c	4009**	68.4	cdefg	42.2	c	31.1	d	4010**	65.6	cdef	37.0	fg	32.1	ef
1724	59.8	k		9.3	k	7.0	k								4011**	65.9	cdef	43.8	d	40.9	c
															4012**	57.0	lm	22.7	lm	41.9	c
															4013*	63.7	efghi	27.7	k	17.8	ij
															4014*	63.3	fghi	37.6	fg	18.3	hij

source: Kisoro = 1000 +, Kabale = 2000 +, Mbarara = 3000 +, Mbale = 4000 +; colony diameter = measured diameter on media minus inoculating plug (5mm)

*, ** = Intermediate and resistant *P. infestans* isolates in at least one run of the experiment

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using Ls means PDF.

Table 12 Reaction of 80 *P. infestans* isolates from different parts of Uganda to metaxyl, 1998-99.

Reaction	Month and year			Totals	
	May 1998	Nov. 1998	May 1999	No.	%
Resistant	11(2.8)	8 (2.0)	17(4.3)	36(9.0)	44.4
Intermediates	7 (1.8)	7 (1.8)	5 (1.3)	19(4.8)	23.5
Sensitive	7 (1.8)	11(2.8)	8 (2.0)	26(6.5)	32.1
Total	25	26	30	81	100

Means in parenthesis within row and column are not significantly different at $P \leq 0.05$ using Ls means probability different (PDF), after square root transformation ($x + 0.5$). Resistant: growth on media with 5µg/ml and 100µg/ml metaxyl $\geq 40\%$ of 0µg/ml;

Intermediates: growth on media with 5µg/ml metaxyl $\geq 40\%$ of 0µg/ml;

Sensitive: growth on media with 5µg/ml and 100µg/ml metaxyl $\leq 40\%$ of 0µg/ml.

Table 13 Mean squares of transformed numbers of Metaxyl reaction of different *P. infestans* isolates from different locations of Uganda, 1998-1999

Source	DF	Mean square
Season	2	ns
Reactions	2	0.4 ns
District	3	1.3*
Reaction x District	4	0.5 ns
Error	22	0.4

ns, * denotes not significant and significant at $P \leq 0.05$. Numbers transformed using squareroot transformation ($\sqrt{x+0.5}$)

Table 14 Metaxyl reaction of different *P. infestans* isolates from each district during 1998-99

District	Reaction	
	Resistant	Intermediate
	No	No
	%	%
Kisoro	1 (0.3) c	3 (1.0) c
Kabale	18(6.0) a	5 (1.7) bc
Mbarara	9 (3.0) abc	6 (2.0) ab
Mbale	8 (2.7) abc	5 (1.7) bc
Total	36	19
	100.0	100.0
		26
		100.0

Means in parenthesis within column followed by similar letters are not significantly different at $P \leq 0.05$ using LS means probability different(PDF), after square root transformation ($\sqrt{x+0.5}$). Resistant: growth on media with 5µg/ml and 100µg/ml metaxyl $\geq 40\%$ of 0µg/ml; Intermediate: growth on media with 5µg/ml metaxyl $\geq 40\%$ of 0µg/ml; Sensitive: growth on media with 5µg/ml and 100µg/ml metaxyl $\leq 40\%$ of 0µg/ml.

4.3.3 Mating type test

Oospore production (Plate 3) was inconsistent and as such data for individual experiment repeats are presented. The number of oospores produced varied significant ($P < 0.05$) with the isolates tested, whether it was under mating or selfing (Table 15). Among the isolates collected in May 1998 (Table 16), isolates 2002, 3008 and 4001 produced the highest number of oospores in matings, averaging, respectively, 12.0, 26.0 and 17.5 oospores. However, production of oospores was inconsistent. For example, isolate 3008 produced 15 oospores in the first test, 12 in the second, but 51 in the third. Contrastingly, isolate 3012 produced 15 oospores in the first test, six in the second and 10 in the third. Isolate 3013 consistently produced the highest number of oospores in selfing and only few in matings. Isolate 1001, 3004, 3007 and 3011 did not produce oospores, whether in matings or selfing (Table 16).

For the November 1998 isolates (Table 17), isolates 1007, 2017, 2018, 3016 3017 and 3021 produced the largest number of oospores. Isolates 1007, 3017 and 3020 produced large number of oospores in both mating and selfing. Isolates 2006, 2008, 2013, 2015, 2021 and 2023 did not produce oospores.

Among the isolates collected in May 1999 (Table 18), isolate 3022 produced many oospores, but only under mating. Isolates 2030, 2036, 2046, 3021 and 4012 produced oospores in mating and selfing, but with more oospores under mating. For this season, isolates 2025, 2028, 2032 and 2033 did not produce oospores whether in mating or selfing.



Plate 3. An oospore produced by *P. infestans* in mating and selfing tests

Table 15 Mean squares of transformed(T) oospore numbers of *P. infestans* isolates in selfing and mating for the different collections

Source	DF	1st run	2nd run	3rd run	Mean
May 1998 Collection					
Replication	3	1.7*	0.7 ns	4.3*	4.3*
Isolates	25	5.0**	2.3**	8.5**	8.5**
Mating type tests (MTS)	1	13.9**	14.8**	25.3**	25.3**
Isolates x MTS	25	4.3**	1.7**	4.4**	4.3**
Error	153	0.5	0.3	1.2	1.2
November 1998 Collection					
Replication	3	4.2*	3.7 ns	0.4 ns	1.7 ns
Isolates	25	22.7**	15.0**	11.1**	16.9**
Mating type tests (MTS)	1	8.2*	8.7*	0.2 ns	0.004 ns
Isolates x MTS	25	3.7**	8.0**	9.5**	6.4**
Error	153	1.4	1.6	0.8	0.7
May 1999 Collection					
Replication	3	2.0*	3.2*	4.0*	40.3**
Isolates	28	1.9**	2.7**	3.8**	3.1**
Mating type tests (MTS)	1	1.5 ns	9.0*	0.03 ns	2.9*
Isolates x MTS	28	2.6**	2.4*	3.3**	2.5**
Error	171	0.7	1.2	1.3	0.6

ns, * and ** denote not significant and significant at $P \leq 0.05$ and $P \leq 0.001$, respectively

Table 16 Oospore production by *P. infestans* isolate in selfing and mating tests for the May 1998 collection

Isolate	Mean number of oospores per plate					
	1st run		2nd run		3rd run	
	Mating	Selfing	Mating	Selfing	Mating	Selfing
1001	0 (0.7) g 0	0 (0.7) f	0 (0.7) g	0 (0.7)	0 (0.7) d	0 (0.7)
1002	4 (2.1) cdef	0 (0.7) f	1 (1.2) efg	0 (0.7)	1 (1.2) d	0 (0.7)
1003	1 (1.2) fg	0 (0.7) f	1 (1.2) efg	1 (1.2)	1 (1.2) d	0 (0.7)
2001	9 (2.9) abc	1 (1.2) ef	3 (1.9) cde	5 (2.3) a	2 (1.6) cd	2 (1.6)
2002	6 (2.4) cd	0 (0.7) f	15(3.7) a	0 (0.7)	14 (3.8) b	2 (1.6)
2003	9 (2.9) abc	0 (0.7) f	3 (1.9) def	1 (1.2)	2 (1.6) cd	0 (0.7)
3001	3 (1.8) def	0 (0.7) f	1 (1.2) efg	0 (0.7)	3 (1.9) cd	0 (0.7)
3002	6 (2.4) cde	1 (1.2) ef	2 (1.6) def	1 (1.2)	13(3.7) b	1 (1.2)
3003	6 (2.4) cde	0 (0.7) f	2 (1.6) def	1 (1.2)	2 (1.6) cd	5 (2.3)
3004	0 (0.7) g 0	0 (0.7) f	0 (0.7) g 0	0 (0.7)	0 (0.7) d	0 (0.7)
3005	12(3.4) ab	5 (2.3) cd	9 (3.1) b	1 (1.2)	1 (1.2) cd	1 (1.2)
3006	3 (1.8) def	0 (0.7) f	2 (1.6) def	0 (0.7)	6 (2.6) bcd	0 (0.7)
3007	0 (0.7) g 0	0 (0.7) f	0 (0.7) g 0	0 (0.7)	0 (0.7) d	0 (0.7)
3008	15(3.9) a	3 (1.8) cde	12(3.5) ab	1 (1.2)	51(7.2) a	2 (1.6)
3009	4 (2.1) cdef	11(3.4) b	2 (1.6) def	0 (0.7)	5 (2.3) bcd	4 (2.1)
3010	2 (1.6) efg	0 (0.7) f	1 (1.2) efg	0 (0.7)	3 (1.9) d	0 (0.7)
3011	0 (0.7) g 0	0 (0.7) f	0 (0.7) g 0	0 (0.7)	0 (0.7) d	0 (0.7)
3012	15(3.9) a	0 (0.7) f	6 (2.6) cd	0 (0.7)	10(3.2) bc	1 (1.2)
3013	2 (1.6) efg	44(5.9) a	1 (1.2) efg	10(3.2) a	9 (3.1) bc	47(6.9) a
4001	7 (2.6) bcd	4 (2.0) cde	8 (2.9) bc	2 (1.6)	39(6.3) a	5 (2.3)
4002	7 (2.7) bcd	0 (0.7) f	3 (1.9) def	0 (0.7)	1 (1.2) d	0 (0.7)
4003	4 (2.1) cdef	6 (2.4) bc	2 (1.6) def	0 (0.7)	3 (1.9) cd	2 (1.6)
4004	2 (1.3) efg	5 (2.0) cde	1 (1.2) fg	2 (1.6)	2 (1.6) d	2 (1.6)
4005	0 (0.7) g 3	1 (1.8) cdef	0 (0.7) g 0	0 (0.7)	1 (1.2) d	1 (1.2)
4006	3 (1.8) defg	6 (2.4) cd	2 (1.6) def	2 (1.6)	4 (2.1) bcd	1 (1.2)
1724*	0 (0.7) g 0	0 (0.7) f	0 (0.7) g 0	0 (0.7)	0 (0.7) d 0	0 (0.7)
						0.0 (0.7) d
						2.1 (1.6) d
						1.1 (1.3) d
						5.0 (2.3) cd
						12.0(3.5) b
						4.4 (2.2) cd
						2.3 (1.7) d
						7.0 (2.7) b
						3.2 (1.9) d
						0.0 (0.7) d
						7.0 (2.7) c
						4.0 (2.1) d
						0.0 (0.7) d
						0.0 (0.7) d
						26.0(5.2) a
						4.0 (2.1) d
						2.0 (1.6) d
						0.0 (0.7) d
						0.0 (0.7) d
						2.1 (1.6)
						5.0 (2.3)
						0.0 (0.7)
						0.0 (0.7)
						0.3 (0.9)
						34.0 (5.9)
						17.5(4.2) a
						4.0 (2.1) d
						3.0 (1.9) d
						1.0 (1.2) d
						0.3 (0.9) d
						3.0 (1.9) d
						1.0 (1.2)
						2.3 (1.7)
						0.0 (0.7)

Isolate source: Kisoro=1000+, Kabale=2000+, Mbarara=3000+, Mbale=4000+; 1724* is A1 tester isolate

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using Ls means PD, after square root transformation ($\sqrt{x+0.5}$)

Numbers in parenthesis indicate number of oospores after transformation.

Table 17 Oospore production by *P. infestans* isolates in selfing and mating tests for the November 1998 collection

Isolate	Mean number of oospores per plate					
	1st run		2nd run		3rd run	
	Mating	Selfing	Mating	Selfing	Mating	Selfing
1004	18(4.3) b	9 (3.1) c	5 (2.3) bcd	2 (1.6) b	4 (2.1) cde	2 (1.6) cde
1005	10(3.2) bcd	0 (0.7) d	1 (1.2) d	0 (0.7)	1 (1.2) de	0 (0.7) e
1007	59(7.7) a	78(8.9) a	17(4.2) ab	27(5.2) c	10(3.2) c	6 (2.5) cd
2004	0 (0.7) e 0	(0.7) d	0 (0.7) d	1 (1.2)	0 (0.7) e	2 (1.6) cde
2005	9 (3.1) bcd	0 (0.7) d	5 (2.3) bcd	0 (0.7)	4 (2.1) de	0 (0.7) e
2006	0 (0.7) e 0	(0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	0 (0.7) e
2008	0 (0.7) e 0	(0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	0 (0.7) e
2009	8 (2.9) bcd	0 (0.7) d	4 (2.1) cd	0 (0.7)	2 (1.6) de	1 (1.2) de
2010	5 (2.3) cde	3 (1.8) cd	1 (1.2) d	0 (0.7)	4 (2.1) de	0 (0.7) e
2013	0 (0.7) e	0(0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	1 (1.2) de
2014	0 (0.7) e	0 (0.7) d	3 (1.8) bcd	0 (0.7)	0 (0.7) e	1 (1.2) de
2015	0 (0.7) e	0 (0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	0 (0.7) e
2016	1 (1.2) e	1 (1.2) cd	1 (1.2) d	2 (1.6)	0 (0.7) e	1 (1.2) de
2017	41(6.4) a	8 (2.9) c	23(4.8) a	2 (1.6)	64(8.0) a	2 (1.6) cde
2018	4 (2.1) de	0 (0.7) d	1 (1.2) d	0 (0.7)	48(7.0) b	0 (0.7) e
2021	0 (0.7) e	0 (0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	0 (0.7) e
2022	0 (0.7) e	0 (0.7) d	0 (0.7) d	6 (2.5)	0 (0.7) e	2 (1.6) cde
2023	0 (0.7) e	0 (0.7) d	0 (0.7) d	0 (0.7)	0 (0.7) e	0 (0.7) e
3014	0 (0.7) e	2 (1.6) cd	0 (0.7) d	3 (1.8)	1 (1.2) de	7 (2.7) e
3015	13(3.7) bcd	1 (1.2) cd	5 (2.3) bc	4 (2.1)	4 (2.1) de	2 (1.6) de
3016	3 (1.8) e	9 (3.1) c	2 (1.6) d	60(7.8) ab	2 (1.6) de	26(5.1) b
3017	12(3.5) bcd	33(5.8) b	2 (1.6) d	37(6.1) bc	1 (1.2) de	24(4.9) b
3020	15(3.9) bc	11(3.4) c	9 (3.1) abc	97(9.8) a	6 (2.5) cd	43(6.6) a
4007	0 (0.7) e	0 (0.7) d	0 (0.7) d	3 (1.8)	1 (1.2) e	1 (1.2) de
4008	0 (0.7) e	0 (0.7) d	2 (1.6) d	0 (0.7)	1 (1.2) e	0 (0.7) e
4009	1 (1.2) e	0 (0.7) d	1 (1.2) d	0 (0.7)	0 (0.7) e	0 (0.7) e
					9.0 (3.1) cde	4.1 (2.1) c
					4.1 (2.1) defg	0.0 (0.7) d
					29.0 (5.4) b	46.0 (6.8) ab
					0.0 (0.7) i	1.0 (1.2) cd
					6.0 (2.5) def	0.0 (0.7) d
					0.0 (0.7) i	0.0 (0.7) d
					0.0 (0.7) i	0.0 (0.7) d
					5.0 (2.3) def	0.4 (0.9) d
					3.3 (1.9) eghi	1.1 (1.3) cd
					0.0 (0.7) i	0.0 (0.7) d
					1.0 (1.2) ghi	0.3 (0.9) d
					0.0 (0.7) i	0.0 (0.7) d
					43.0 (6.6) a	5.0 (2.3) c
					18.0 (4.3) c	0.0 (0.7) d
					0.0 (0.7) i	0.0 (0.7) d
					0.0 (0.7) i	3.0 (1.9) cd
					0.0 (0.7) i	0.0 (0.7) d
					0.4 (0.9) hi	4.1 (2.1) c
					7.3 (2.8) cde	2.2 (1.6) cd
					2.0 (1.6) fghi	32.0 (5.7) ab
					5.2 (2.4) def	31.0 (5.6) b
					10.0 (3.2) cd	50.0 (7.1) a
					0.3 (0.9) hi	1.3 (1.3) cd
					1.0 (1.2) ghi	0.0 (0.7) d
					1.0 (1.2) ghi	0.0 (0.7) d

Isolate source: Kisoro = 1000 +, Kabale = 2000 +, Mbarara = 3000 +, Mtale = 4000 +; 1724* is A1 tester isolate

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using Ls means PD, after square root transformation ($\sqrt{x+0.5}$). Numbers in parenthesis indicate means of number of oospores after transformation.

Table 18 Oospore production by *P. infestans* isolates in selfing and mating tests for the May 1999 collection

Isolate	Mean number of oospores per plate					
	1st run		2nd run		3rd run	
	Mating	Selfing	Mating	Selfing	Mating	Selfing
1008	0 (0.7) c	2 (1.6) bc	0 (0.7) c	2 (1.6) b	0 (0.7) d	0 (0.7) c
1009	0 (0.7) c	3 (1.8) bc	0 (0.7) c	11(3.4) a	0 (0.7) d	17(4.2) b
2024	0 (0.7) c	5 (2.3) ab	0 (0.7) c	2 (1.6) b	0 (0.7) d	0 (0.7) c
2025	0 (0.7) c	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2026	0 (0.7) c	3 (1.8) bc	2 (1.6) c	0 (0.7) b	0 (0.7) d	3 (1.8) bc
2027	4 (2.1) bc	1 (1.2) bc	2 (1.6) c	2 (1.6) b	1 (1.2) d	5 (2.3) bc
2028	0 (0.7) c	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2029	0 (0.7) c	1 (1.2) bc	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2030	4 (2.1) bc	6 (2.5) ab	3 (1.8) c	4 (2.1) ab	8 (2.9) abc	16(4.1) b
2031	2 (1.6) bc	0 (0.7) c	3 (1.8) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2032	0 (0.7) c	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2033	0 (0.7) c	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2034	1 (1.2) c	0 (0.7) c	1 (1.2) c	2 (1.6) b	2 (1.6) cd	0 (0.7) c
2035	2 (1.6) bc	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2036	8 (2.9) b	1 (1.2) bc	3 (1.8) c	1 (1.2) b	5 (2.3) bcd	2 (1.6) bc
2037	3 (1.8) bc	0 (0.7) c	4 (2.1) bc	0 (0.7) b	2 (1.6) cd	0 (0.7) c
2038	1 (1.2) c	0 (0.7) c	5 (2.3) bc	0 (0.7) b	9 (3.1) abc	0 (0.7) c
2039	29(5.4) a	0 (0.7) c	6 (2.5) bc	3 (1.8) b	1 (1.2) d	1 (1.2) c
2040	3 (1.8) bc	0 (0.7) c	4 (2.1) bc	0 (0.7) b	4 (2.1) bcd	0 (0.7) c
2042	0 (0.7) c	6 (2.5) ab	0 (0.7) c	4 (2.1) ab	0 (0.7) d	5 (2.3) bc
2043	2 (1.6) bc	0 (0.7) c	0 (0.7) c	0 (0.7) b	0 (0.7) d	0 (0.7) c
2045	0 (0.7) c	1 (1.2) bc	0 (0.7) c	1 (1.2) b	0 (0.7) a	0 (0.7) c
2046	6 (2.5) b	2 (1.6) bc	13(3.6) ab	5 (2.3) ab	19(4.4) a	1 (1.2) c
3021	4 (2.1) bc	5 (2.3) b	13(3.6) ab	4 (2.1) ab	7 (2.7) abc	4 (2.1) bc
3022	3 (1.8) bc	0 (0.7) c	12(3.5) b	0 (0.7) b	17(4.2) ab	0 (0.7) c
4010	4 (2.1) bc	0 (0.7) c	3 (1.8) c	0 (0.7) b	1 (1.2) d	0 (0.7) c
4011	2 (1.6) bc	1 (1.2) bc	18(4.3) a	0 (0.7) b	4 (2.1) bcd	11(3.4) b
4012	3 (1.8) bc	1 (1.2) bc	30(5.5) a	1 (1.2) b	5 (2.3) bcd	6 (2.5) b
4013	1 (1.2) c	11(3.3) a	2 (1.6) c	4 (2.1) ab	0 (0.7) d	30(5.5) a
						1.0 (de)
						16.0 (4.1) a

Isolate source: Kisoro=1000+, Kabale=2000+, Mbarara=3000+, Mbale=4000+; 1724* is A1 tester isolate

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using Ls means PD, after square root transformation ($\alpha + 0.5$)
Numbers in parenthesis indicate means of number of oospores after transformation.

Overall, 82.5% (66 of 80) of the isolates tested produced oospores in matings and selfings with more oospore production in mating than selfing (Table 19). On average the number of oospores encountered ranged from 1 to about 90 per plate. For the 80 *P. infestans* tested 50.0 % of the isolates produced oospores in matings and selfings, 22.5% in mating only and 10% in selfing only. About 18% of the isolates did not produce oospores in the two tests. Table 20 and 21 show that oospore production was not influenced by seasonal difference, but were significantly influenced by the district of origin. The number of *P. infestans* isolates that produced oospores in both mating and selfing was consistently higher (average 13.3) across all the seasons tested, while selfing alone produced the lowest (average 2.7).

The number of isolates that produced oospores varied significantly ($P \leq 0.05$) with locations. Although the greatest number of isolates that produced oospores fall in the group of both mating and selfing reaction, Kabale district recorded the highest number of isolates in each of these categories (Table 21). Interestingly, Kabale also had the largest number of isolates that did not produce oospores. All isolates from Mbale produced oospores.

4.3.4 Relationship between metalaxyl sensitivity and oospore production

The isolates used for metalaxyl tests were the same isolates used for oospore production. Table 22 shows the relationship between metalaxyl resistance and oospore production. Ninety two percent (33 of 36) of the highly resistant *P. infestans* isolates produced

Table 19 Production of oospores by *P. infestans* in mating and selfing for Uganda isolates collected in the period 1998-99

Production of oospores in	Number of isolates	% of the total isolates
Non oospore producers	14	17.5
Selfing only	8	10.0
Mating only	18	22.5
Both mating and selfing	40	50.0
Total	80	100.0

Based on 3 collections, May 1998, November 1998 and May 1999

Table 20 Mean squares of transformed numbers of *P. infestans* isolates from different districts of Uganda which produced oospores in May 1998, November 1998 and May 1999

Source	DF	Mean square
Districts	3	1.4*
Production of oospores (PO)	3	1.7*
Seasons	2	ns
PO x District	6	1.6 ns
Error	33	0.3

ns, * denotes not significant and significant at $P \leq 0.05$. Numbers transformed using square root transformation ($x+0.5$)

Table 21 Oospore production by *P. infestans* isolates from different parts of Uganda, 1998-99

Production of oospores in	Kisoro	Kabale	Mbarara	Mbale	Overall
No oospores	1 (0.3) cd	10(3.3) abc	3 (1.0) cd	0 (0.0)	14
Selfing only	2 (0.7) cd	6 (2.0) abcd	0 (0.0) d	0 (0.0)	8
Mating only	2 (0.7) cd	8 (2.7) abcd	4 (1.3) bcd	4 (1.3)	18
Both mating & selfing	3 (1.0) bcd	15(5.0) a	13(4.3) ab	9 (3.0)	40
Total	8	39	20	13	80

Means in parenthesis within columns are significantly different at $P \leq 0.05$ using Ls means probability different(PDF), after square root transformation ($x+0.5$).

oospores in cultures. On average the number of oospores produced by highly resistant isolates ranged between 1 to 50 per plate. Of the 36 isolates, 11 (30.5% of metalaxyl resistant isolates) produced oospores in mating only and 20 (55.5%) produced oospores in both mating and selfing. Three metalaxyl resistant isolates (8.3%), namely 2021, 2028, 2033 did not produce oospores in both tests (Table 22).

Eighty three percent (15 of 18) of the intermediary resistant isolates produced oospores in cultures, with 1 to 45 oospores per plate. Sixty seven percent of the intermediary resistant isolates (12 of 18) produced oospores in both mating and selfing tests. Six percent (1 of 18) of intermediate resistant isolates produced oospores in selfing only, 11.1%(2 of 18) in mating only and 16.7% (3 of 18) did not produce oospores (Table 22). The three intermediary resistant isolates that did not produce oospores in cultures were; 3004, 2025 and 2032.

Among the metalaxyl sensitive *P. infestans* isolates, 69.2% (18 of 26) produced oospores in cultures, with 1 to 20 oospores per plate. Only 5 (19.2%) of the sensitive isolates produced oospores in mating only, 5 (19.2%) in selfing only, and 8 (30.7%) in both selfing and mating. Eight (30.7%) of the sensitive isolates that did not produce oospores; the isolates included; 1001, 3007, 3011, 2006, 2008, 2013, 2015 and 2023. Overall, irrespective of the metalaxyl sensitivity, most of the oospores were produced by the metalaxyl resistant isolates, followed by sensitive isolates; the least number of oospore production was by intermediate category (Table 22).

Table 22 Relationship between metalaxyl sensitivity and oospore production by *P. infestans* isolates from Uganda

Metalaxyl reaction	Total No of isolates	Non oospore producers	Oospore producing isolates in		
			Mating	Selfing	Mating & selfing
Resistant	36	3 (0.8) cd	11(2.8) bc	2 (0.5) d	20 (5.0) a
Intermediates	19*	3 (0.8) cd	2 (0.5) d	1 (0.3) d	12 (3.0) ab
Sensitive	26	8 (2.1) bcd	5 (1.3) bcd	5 (1.3) bcd	8 (2.0) bcd
Total	81	14	18	8	40

*one isolate not tested for mating type test. Resistant: growth on media with 5 µg/ml and 100 µg/m metalaxyl $\geq$ 40% of 0 µg/ml; Intermediates: growth on media with 5 µg/ml metalaxyl $\geq$ 40% of 0 µg/ml; Sensitive: growth on media with 5 µg/ml and 100 µg/ml metalaxyl $\leq$ 40% of 0 µg/ml. Means in parenthesis within row and column with similar letters do not differ significantly $P \leq 0.05$ using Ls mean PD. Means in parathesis represent average number of isolates from each district.

4.3.5 Virulence of *P. infestans* isolates on potato and tomato leaflets

A total of 61 *P. infestans* isolates recovered from potato were tested for their virulence on potato and tomato detached leaflets. The amount of leaf damage (lesion size and mean percentage area blighted) varied significantly ($P < 0.05$) with the isolates. Lesion sizes and percentage area blighted varied significantly ($P \leq 0.05$) with the inoculated hosts (tomato and potato)(Tables 23).

Among the 20 isolates tested from the May 1998 collection (Table 24), all the isolates caused disease on detached potato leaves. Lesion sizes (length) formed ranged from 1.9 mm for isolate 2003 to 28 mm for isolate 3010. Percentage area blighted ranged from 11.3 to 51.3% for isolates 2003 and 3008, respectively. Nine of the 20 isolates did not cause disease on detached tomato leaves. Those that caused disease on tomato included, 2002, 2003, 3008, 4001, 4001, 4002, 4003, 4004 and 4006, with lesion size ranging from 9.0 mm for isolate 3010 to 22.5 mm for isolate 4002. The corresponding

percentage area blighted ranged from 7.8 to 32.5% for isolates 3010 and 4002, respectively. Isolates 4002 and 4004 caused significantly ($P \leq 0.05$) more disease on the detached tomato leaves than on potato as indicated by the bigger lesion sizes on tomato of 22.5 and 18.8 mm, respectively, compared to 14.3 and 16.8 mm on potato. Percentage area affected were 32.5 and 26.7 on tomato for isolates 4002 and 4004, respectively, compared to 20.0 and 24.6% on potato leaves (Table 24).

Eighteen isolates were tested for virulence on potato and tomato from the November 1998 collection (Table 25). A total of 11 isolates caused lesions on tomato leaflets. Isolate 2009 caused the least lesion size of 9.3 mm and isolate 4008 the biggest (31.0 mm). The least percentage area affected was 8.0 mm and the highest 36.3% for isolates 2009 and 2015, respectively. The biggest percentage area blighted was 21.1 for isolate 2016, and the least 3.2% for isolate 2005. Of the 11 isolates that caused disease on tomato leaflets, isolates 2009, 2014, 2015, 2016, 2018, 2021 and 3020 caused lesion sizes of more than 10 mm, and were, therefore, considered to have infected tomato (Plate 4). Overall, however, lesion sizes and percentage infected area on tomato leaflets were smaller than those on potato leaflets (Table 25).

For the May 1999 collection, 23 isolates were tested for virulence on potato and tomato leaflets (Table 26). The biggest lesion size of 25.0 mm on potato leaflets was produced by isolate 2025 with an affected area of 30%; the smallest lesion size 13.8 mm was caused by isolate 3022. On tomato leaflets isolate 2046 produced the biggest lesion of 23.8 mm, with 30.6% affected leaf area, while 2029 produced the smallest size of 1.1 mm. Of the tested isolates 12 infected tomato causing lesion size of more than 10 mm.



Plate 4. Tomato leaflets infected (black lesions) with a *P. infestans* isolate from potato

Virulence of the *P. infestans* isolates was also related to metalaxyl sensitivity, oospore production and origin of the isolate. Of the 61 *P. infestans* isolates used for the virulence test, 14 (22.9%) that infected detached tomato leaves were highly resistant to metalaxyl (Table 27), 5 (8.2%) intermediates, and 8 (13.1%) sensitive. Thus, a total of 27 (44.2%) isolates infected tomato leaves. All the isolates caused disease on potato leaves except isolate 2009 which caused a lesion size of less than 10 mm.

Table 28 shows that 18 (29.5%) of the isolates tested that infected tomato leaflets produced oospores in both mating and selfing, 2 (3.3%) in selfing only, 3 (4.9%) in mating only, and 3 (4.9%) did not produce oospores. Irrespective of oospore production, all the isolates caused disease on detached potato leaflets.

Considering location, 16 (26.2%) of the isolates that were virulent on tomato leaves originated from Kabale, and 7 (11.5%) and 3 (5%) from Mbale and Mbarara, respectively. Only 1 isolate virulent on tomato leaves was from Kisoro. Overall, the largest number of isolates that infected tomato leaves were from the highly resistant metalaxyl group of *P. infestans* isolates, and produced oospores in either mating or selfing or both (Tables 27 & 28). Most of these isolates originate from Kabale and Mbale districts (Table 29).

Table 23 Mean squares of transformed lesion length and % affected area affected by *P. infestans* isolates collected in May and November, 1998 and May 1999, on detached leaves of potato and tomato

Source	1st run		2nd run		3rd run		Mean	
	DF	Lesion length Blighted area (%)	DF	Lesion length Blighted area (%)	DF	lesion length Blighted area (%)	DF	Lesion length Blighted area
May 1998 collection								
Replication	3	0.7* 69.5*	3	0.2 ns 37.3 ns	3	0.1 ns 5.4 ns	3	0.2 ns 17.9*
Isolates	20	4.7** 3004.6**	14	9.8** 527.7**	14	6.6** 355.9**	20	6.0** 349.6**
Hosts	1	173.2** 13218.4**	1	106.7**4579.8**	1	99.4** 2888.7**	1	172.0** 10442.3**
Isolate x Host	20	6.6** 416.4**	13	4.7** 216.8**	13	4.0** 157.1**	20	5.0** 301.8**
Error	123	0.2 18.4	82	0.1 15.5	82	0.05 5.6	123	0.08 6.3
November 1998 collection								
Replication	3	0.3 ns 89.4*	3	0.05 ns 4.2 ns	3	5.0** 4.5 ns	3	3.8* 10.9*
Isolates	17	9.6** 694.4**	15	7.1** 312.9**	15	72.6** 337.0**	17	7.6** 179.5**
Hosts	1	212.2**15448.4**	1	150.5**714.5**	1	95.9** 6220.8**	1	191.8** 4382.4**
Isolate x Host	17	5.4** 282.5**	14	5.5** 241.7**	14	5.9** 263.5**	17	4.2** 96.7**
Error	105	0.2 17.6	87	0.1 8.3	87	0.8 7.4	105	0.06 3.9
May 1999 collection								
Replication	3	0.04 ns 6.0 ns	3	0.06 ns 1.3 ns	3	0.02 ns 3.2 ns	3	0.01 ns 1.0 ns
Isolates	22	8.4** 419.2**	20	6.1** 301.2**	13	5.2** 168.1**	22	6.8** 291.3**
Hosts	1	144.2** 6029.6**	1	126.7**3650.3**	1	159.9**4549.2**	1	148.9** 4977.4**
Isolate x Host	22	5.8** 293.9**	20	5.3** 245.2**	13	6.5** 253.0**	22	5.4** 221.8**
Error	135	0.14 13.7	123	0.2 13.2	81	0.09 7.0	135	0.07 5.7

ns, * and ** denote not significant, and significant at $P \leq 0.05$ and $P \leq 0.001$, respectively
 Lesion length underwent square root transformation ($\sqrt{x+0.5}$), % area affected Arcsin transformation ($\sqrt{x+1}$)

Table 24 Virulence of *P. infestans* isolates collected in May 1998 on detached leaves of potato and tomato

Isolate	Potato		Tomato	
	Lesion length (mm)	Area blighted (%)	Lesion length (mm)	Area blighted (%)
1001	17.2±0.4 (4.2) def	20.4±2.1 (27.6) jlk	0.0±0.0 (0.7)	0.0±0.0 (5.7)
1002	17.4±1.2 (4.2) ef	23.2±2.8 (29.5) hij	0.0±0.0 (0.7)	0.0±0.0 (5.7)
1003	16.0±1.4 (4.1) efg	30.0±4.1 (33.8) efg	0.0±0.0 (0.7)	0.0±0.0 (5.7)
2002*	21.9±2.4 (4.7) bc	40.4±5.5 (40.1) c	19.3±3.4 (4.2) ab	25.8±5.7 (31.1) b
2003*	11.9±2.9 (3.5) i	11.3±3.1 (20.3) m	15.3±3.0 (3.9) c	17.9±1.5 (25.8) c
3001	21.3±2.2 (4.6) cd	37.5±2.8 (38.3) cd	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3002	21.3±4.3 (4.6) cd	30.0±4.1 (33.8) efg	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3003	15.2±4.4 (3.9) fgh	19.2±2.8 (26.6) jk	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3006	12.6±2.3 (3.6) hi	10.8±1.7 (20.1) m	2.7±0.5 (1.8) f	2.3±0.7 (10.3) g
3007	13.8±1.1 (3.8) ghi	14.2±3.2 (22.8) lm	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3008*	26.4±4.8 (5.2) a	51.3±13.2 (46.3) a	10.3±1.7 (3.2) d	7.0±2.4 (16.3) ef
3009	18.8±1.7 (4.4) cde	23.8±4.7 (29.7) hij	5.5±1.7 (2.4) e	4.0±1.5 (12.8) fg
3010	28.0±2.1 (5.3) a	47.5±2.9 (44.2) b	9.0±1.4 (3.1) d	7.8±2.6 (17.1) e
3011	12.3±2.7 (3.5) hi	19.2±7.7 (26.4) jk	2.3±2.6 (1.5) f	2.9±3.4 (10.4) g
4001*	12.8±2.2 (3.6) hi	17.1±3.7 (25.1) kl	11.1±1.9 (3.4) d	16.2±3.4 (24.4) cd
4002*	14.3±2.4 (3.9) fghi	20.0±4.1 (27.2) jlk	22.5±3.0 (4.8) a	32.5±2.9 (35.4) a
4003*	21.7±2.1 (4.7) bc	31.7±4.7 (34.8) def	11.0±0.6 (3.4) d	12.1±2.1 (21.2) d
4004*	16.8±2.4 (4.1) efg	24.6±3.7 (30.4) ghi	18.8±2.2 (4.4) ab	26.7±3.7 (31.7) b
4005	24.9±2.7 (5.0) ab	32.9±3.2 (35.6) de	0.0±0.0 (0.7)	0.0±0.0 (5.7)
4006*	22.2±1.5 (4.8) bc	26.3±2.9 (31.4) fgh	16.9±1.7 (4.2) bc	20.0±0.0 (27.3) c
*1724	11.8±0.6 (3.5) i	13.3±1.3 (22.2) lm	0.0±0.0 (0.7)	0.0±0.0 (5.7)

* *P. infestans* isolates that infected tomato detached leaves; *1724 is A1 isolate from Kenya

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using LS means PD, after square root transformation ($x+0.5$) for lesion length and Arcsin transformation ($x+1$) for percentage affected area.

Numbers in parenthesis indicate means of lesion length and percentage affected area after transformation.

-Pooled data for 3 experimental repeats

Table 25 Virulence of *P. infestans* isolates collected in November 1998 on detached leaves of potato and tomato

Isolate	Potato		Tomato	
	Lesion length (mm)	Area blighted (%)	Lesion length (mm)	Area blighted (%)
1004	22.7±0.7 (4.8) bc	19.4±0.9 (26.8) def	0.0±0.0 (0.7)	0.0±0.0 (5.7)
2005	19.9±1.7 (4.5) cd	18.1±2.1 (25.9) efg	3.0±1.0 (1.8)i	3.2±1.0 (11.9)
2008	23.4±2.3 (4.9) b	19.4±1.6 (26.8) def	9.0±2.1 (3.1)f	10.7±2.4 (19.9) cd
2009*	9.3±1.0 (3.1) g	8.0±1.8 (17.4) i	12.5±3.1 (3.6)e	11.1±2.9 (20.3) cd
2010	17.9±1.3 (4.3) e	16.5±1.7 (24.8) fgh	3.9±0.9 (2.1)g	3.5±0.8 (12.3)
2014*	22.9±1.7 (4.8) bc	16.9±2.8 (25.0) fgh	15.8±3.9 (4.0)cd	13.2±3.5 (22.0) c
2015*	22.0±4.8 (4.7) bcd	36.3±11.0 (37.5) a	12.5±1.7 (3.6)e	10.0±4.1 (19.2) d
2016*	29.7±2.1 (5.5) a	26.9±3.7 (31.9) b	19.8±1.3 (4.5)b	21.1±2.8 (28.0) a
2017	11.9±3.2 (3.5) g	13.9±5.1 (22.5) h	3.6±0.8 (2.3)h	5.0±1.3 (14.2) e
2018*	24.4±2.4 (5.0) b	19.0±1.9 (26.6) defg	13.4±1.3 (3.7)de	9.8±2.2 (19.2) d
2021*	28.9±4.2 (5.4) a	24.4±1.6 (30.3) bc	16.6±2.4 (4.1)c	16.9±0.8 (25.1) b
2022	25.3±0.7 (5.1) b	21.5±2.1 (28.3) cde	0.0±0.0 (0.7)	0.0±0.0 (5.7)
2023	12.4±0.6 (3.6) fg	15.7±3.0 (24.1) gh	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3014	19.9±1.5 (4.5) cde	19.0±1.3 (26.6) defg	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3015	15.5±6.0 (3.9) f	15.0±4.0 (23.5) h	0.0±0.0 (0.7)	0.0±0.0 (5.7)
3020*	24.7±1.1 (5.0) b	19.9±1.0 (27.2) def	23.5±1.8 (1.8)a	20.7±0.0 (27.7) ab
4007	19.2±1.6 (4.4) de	15.7±0.0 (24.1) gh	0.0±0.0 (0.7)	0.0±0.0 (5.7)
4008	31.0±2.1 (5.6) a	22.8±1.2 (29.2) bcd	0.0±0.0 (0.7)	0.0±0.0 (5.7)

* *P. infestans* isolates that infected tomato detached leaves:

Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using L_s means PD , after square root transformation ($x+0.5$) for lesion length and Arcsin transformation ($x+1$) for percentage affected area.

Numbers in parenthesis indicate means of lesion length and percentage affected area after transformation.

-Pooled data for 3 experimental repeats

Table 26 Virulence of *P. infestans* isolates collected in May 1999 on detached leaves of potato and tomato

Isolate	Potato		Tomato	
	Lesion length (mm)	Area blighted (%)	Lesion length (mm)	Area blighted (%)
1008	17.5±0.6 (4.3) ef	18.8±1.6 (26.4) cd	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
1009*	19.1±1.4 (4.4) def	18.8±1.6 (26.4) cd	18.1±3.4 (4.3) cd	21.3±2.1 (28.2)b
2024	18.2±1.6 (4.3) ef	18.8±1.6 (26.4) cd	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
2025*	25.0±1.6 (5.0) a	30.0±2.8 (33.8) a	14.9±1.9 (3.9) def	15.0±2.0 (23.6)de
2026	17.0±2.0 (4.1) f	18.8±2.5 (26.4) cd	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
2027*	24.6±1.8 (4.9) ab	27.5±2.0 (32.3) a	17.6±0.6 (4.3) cd	13.8±1.4 (22.6)de
2028	16.6±1.8 (4.2) f	15.0±1.3 (23.6) de	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
2029	18.3±1.5 (4.3) ef	17.5±0.9 (25.5) cde	1.1 ±1.2 (1.2) i	6.7 ±1.3 (16.1)f
2030*	19.6±1.9 (4.5) cdef	19.6±2.0 (27.0) c	15.7±1.4 (4.0) de	16.2±3.4 (24.5)cde
2033	20.0±0.9 (4.6) bcde	25.6±3.0 (31.0) ab	2.3 ±2.2 (1.6) h	1.4 ±1.4 (8.6)g
2034*	17.7±0.5 (4.3) ef	18.8±1.6 (26.4) cd	17.3±1.2 (4.2) cd	18.3±1.3 (26.1)bcd
2036*	20.5±1.2 (4.5) cde	22.1±2.1 (28.7) bc	17.2±1.6 (4.2) cd	21.3±4.0 (28.1) b
2037	13.0±2.6 (3.7) g	15.0±2.0 (23.6) de	8.1 ±1.0 (2.9) g	3.1 ±0.8 (11.7) g
2038*	20.5±2.1 (4.6) cde	20.0±4.1 (27.2) c	12.5±3.0 (3.6) f	12.5±2.8 (21.5) e
2040	22.9±0.4 (4.8) abc	25.4±1.6 (30.9) ab	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
2045*	21.9±1.0 (4.8) abcd	27.5±5.2 (32.2) a	19.2±3.2 (4.4) bc	30.4±7.5 (34.0) a
2046*	24.6±1.8 (5.0) ab	29.4±5.5 (33.4) a	23.8±1.0 (4.9) a	30.6±5.1 (34.2) a
3021*	19.7±1.2 (4.5) cdef	19.6±0.9 (27.0) cd	13.8±1.9 (3.8) ef	15.2±4.3 (23.6) de
3022	13.8±1.8 (3.8) g	13.8±1.4 (22.6) e	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
4010	23.1±3.8 (4.8) abc	27.5±7.2 (32.2) a	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
4011*	17.4±0.4 (4.3) ef	17.9±0.8 (25.8) cd	15.8±2.3 (4.1) cd	20.0±3.3 (27.3) bc
4012	22.9±2.2 (4.9) abc	26.7±3.0 (31.7) ab	0.0 ±0.0 (0.7)	0.0 ±0.0 (5.7)
4014*	20.1±3.8 (4.5) cdef	20.0±4.1 (27.2) c	22.3±5.6 (4.7) ab	29.4±3.7 (33.0) a

* *P. infestans* isolates that infected tomato detached leaves. Within columns, means with the same letter(s) and no letters do not differ significantly at ($P \leq 0.05$) using *Ls* means *PD*, after square root transformation ($x+0.5$) for lesion length and Arcsin transformation ($x+1$) for percentage affected area. Numbers in parenthesis indicate means of lesion length and percentage affected area after transformation. Pooled data for 3 experimental repeats

Table 27 Relationship between metalaxyl sensitivity and virulence of *P. infestans* isolates on detached potato and tomato leaflets

Reaction	Total isolates	Number of isolates that infected	
		Potato	Tomato
Resistant	36 (28)	27	14
Intermediates	19 (12)	12	5
Sensitive	26 (21)	21	8
Total	81 (61)	60	27

Numbers in parenthesis indicate the number of isolates used in the virulence test; Resistant: growth on media with 5µg/ml and 100µg/m metalaxyl $\geq$ 40% of 0µg/ml; Intermediates: growth on media with 5µg/ml metalaxyl $\geq$ 40% of 0µg/ml; Sensitive: growth on media with 5µg/ml and 100µg/m metalaxyl $\leq$ 40% of 0µg/ml.

Table 28 Relationship between oospore production and virulence of *P. infestans* isolates on detached potato and tomato leaflets

Oospore production	Total isolates	Number of isolates that infected	
		Potato	Tomato
No oospore producers	14 (10)	10	3
Selfing only	8 (6)	6	2
Mating only	18 (13)	13	3
Mating and selfing	40 (31)	30	18
Not tested	1 (1)	1	1
Total	81 (61)	60	27

Numbers in parenthesis indicate the number of isolates used in the virulence test

Table 29 Relationship between origin of *P. infestans* isolate and virulence of *P. infestans* isolates on detached potato and tomato leaflets

Location	Total isolates	Number of isolates that infected	
		Potato	Tomato
Kisoro	8 (6)	6	1
Kabale	39 (29)	28	16
Mbarara	20 (14)	14	3
Mbale	14 (12)	12	7
Total	81 (61)	60	27

Numbers in parenthesis indicate the number of isolates used in the virulence test

4.4 Discussion

4.4.1 Isolation of *P. infestans*

None of the 10 tomato samples infected with late blight yielded *P. infestans* when isolated with the potato slice method. Yet it is known that potato slices could be infected with local *P. infestans* isolates from tomato samples as reported by Kori (1972). Probably the *P. infestans* on the tomato samples constitute a population genetically distinct from the potato isolates used by Kori (1972). Attempts to grow *P. infestans* from tomato through the tomato fruit yielded two *P. infestans* isolates, which could not grow well or be maintained on V8 medium. Growing *P. infestans* from tomato through tomato fruit as a host tissue is a good approach for isolating the fungus (*Personal observation*), though CIP isolation procedure recommend use of tomato leaf as a host tissue (Forbes, 1997). In this study V8 was not a good medium for growing or maintaining tomato *P. infestans*. Other substrates, notably, rye and pea, have been used elsewhere (Hartman and Huang, 1995; Fraser *et al.*, 1999).

Relatively few numbers of *P. infestans* isolates were recovered from samples collected from Kisoro and Mbale district (Table 9). This was due to the high incidence of bacteria (*R. solanacearum*) on these samples. Notwithstanding the above, the number of *P. infestans* isolates recovered from the samples were generally low across all seasons and districts. Apart from bacteria making it difficult to isolate the fungus, on many occasions the fungus did not grow through the host tissue. Failure of growth may have been due to the fungicides that were applied to the crop before the samples were picked,

resulting in death of the fungus. On a number of occasions it was difficult to come across potato fields that had not been sprayed at all with fungicides. Other earlier studies (eg. Adipala, 1999) also eluded to high frequencies of pesticide usage in these areas. A good number of *P. infestans* isolates were recovered from samples collected from Kabale in May 1999. During this period it was relatively dry, and there was very little or no fungicide usage on the crops.

4.4.2 Metalaxyl resistance

Generally, metalaxyl reduced growth rates of the tested isolates. For a few isolates, growth on 100 μ g/ml of metalaxyl was better than 5 μ g/ml. Also, isolate 2002 grew better on media amended with fungicides than without. Zhang *et al.* (1997) also found metalaxyl resistant isolates of *P. infestans* that grew better on the fungicide at higher concentration than with less concentration or without. The basis for metalaxyl stimulation of growth is unknown. While studying antibiotic resistance in *P. infestans* Shattock and Shaw (1975) observed a similar stimulation of growth by an inhibitory compound, where a streptomycin resistant mutant of *P. infestans* only grew on solid medium containing streptomycine. One possible mode for the stimulation of growth by metalaxyl is through the molecule's interaction with RNA polymerase (Davidse *et al.*, 1983b). In metalaxyl-resistant isolates may have resistance due to altered RNA polymerase I or RNA polymerase III. In metalaxyl stimulated isolates, the target RNA polymerase may be altered such that binding metalaxyl increases enzyme activity.

The most striking and unexpected aspects of this study are the relatively high proportion of metalaxyl resistance among the *P. infestans* isolates collected in the major potato growing districts of Uganda. In this country there is only moderate fungicide application of ridomil to control late blight, largely due to economic limitations (Anonymous, 1998). The survey results reported in chapter three also indicate only moderate use of the fungicide ridomil by potato farmers in Kabale and Kisoro. In Mbale and Mbarara only Dithane M-45 (Mancozeb 80% WP) appeared to be in use.

Half of the highly resistant isolates were from Kabale district. Three *P. infestans* isolates from samples collected at Kalengyere Research Station were highly resistant to metalaxyl. In Kabale, particularly Kalengyere, ridomil is used to control late blight. In other parts of the world, resistance to metalaxyl and other related compounds has developed rapidly in populations of *P. infestans* after the use of metalaxyl to control late blight (Davidse *et al.*, 1983a; Deahl *et al.*, 1995). According to some authors, a greater frequency of resistance to the highly effective phenylamide fungicide metalaxyl occurs among the new biotypes having both A1 and A2 mating types (Fry *et al.*, 1993; Erwin and Ribeiro, 1996). Low dosage of metalaxyl also tends to give high frequencies of metalaxyl resistant *P. infestans* isolates (Davidse *et al.* 1981; Dowley and O'Sullivan, 1981; Davidse *et al.* 1983a).

The high frequency of metalaxyl resistance in *P. infestans* isolates from Kabale points to the possibility that new biotypes of *P. infestans* may have emerged in Uganda. Highly resistant isolates were also found in samples from other parts of Uganda (Mbarara and Mbale), which are known to use less or no ridomil at all to control late blight (see

apter 3). Elsewhere, metalaxyl resistant *P. infestans* isolates were recovered from potato fields where no fungicides were used to produce potato and tomato in North Carolina (Fraser *et al.*, 1999), but the greatest number of *P. infestans* isolates resistant to metalaxyl occurred in fields where metalaxyl was used.

The presence of relatively high number of metalaxyl resistant *P. infestans* in Uganda is not necessarily a strong indicator of the presence of the A2 mating type. This is because there is no genetic correlation between resistance and mating type. It is also known that resistance to phenylamide became established in A1 populations before the appearance of the A2 type (Gisi and Cohen, 1996). Thus, the metalaxyl resistant isolates in Uganda may still be of the A1 mating type.

4.4.3 Mating type test

Some isolates produced oospores in selfing only (10%), others in both mating and selfing (50.0%), mating only (22.5%) and some did not produce oospores in both tests (17.5%). The phenomenon of selfing is common in a number of *Phytophthora* species (Ko, 1988). Interestingly, of the 48 selfing isolates observed in the study, 40 also produced oospores during mating with A1 tester strain. The oospores produced could be as a result of selfing rather than mating. These results are interesting in that they demonstrate selfing in some isolates, and suggest the presence of A2 mating type (i.e., those that produced oospores with the A1 tester). Since no A2 tester isolate was used in the tests, no definite conclusion can be made about the presence of A2 in Uganda based solely on A1 tester isolate. The results also show that the production of oospores in mating and selfing was

not consistent. It is therefore not clear whether the oospores produced during mating are as a result of hybridisation between isolates (mating) or due to selfing. This can only be ascertained after *P. infestans* isolates from Uganda have been tested against both A1 and A2 tester isolates, and by isozyme and DNA finger printing (Old *et al.*, 1984; Goodwin, 1991). Nevertheless, the results point to the existence of varied populations of *P. infestans* in Uganda. Additionally, attempts should be made to examine whether oospores occur in nature in Uganda.

4.4.4 Metalaxyl resistance and oospore production

Results of metalaxyl resistance and mating tests revealed that the isolates that produced the largest number of oospores were those categorised as intermediary or highly resistant to metalaxyl. This is evidence of the possible existence of new *P. infestans* biotypes in Uganda. However, isolates 2021, 2028 and 2033 that were highly resistant to metalaxyl did not produce oospores in the mating tests. If these isolates belong to the A2 mating type, it would confirm the earlier finding that metalaxyl resistance was established in A1 populations before the appearance of A2 (Gisi and Cohen, 1996). Among the isolates studied, 69.2% of the sensitive isolates to metalaxyl produced oospores, but the numbers produced per plate were quite low (1-20 oospores per plate). The low numbers of oospores produced by the sensitive isolates indicate that these may be weak A2s, which probably have not yet developed resistance to metalaxyl or that they are actually A1s which produce oospores by selfing rather than mating. The results indicate that although some *P. infestans* isolates are sensitive to metalaxyl, some have the ability to produce oospores either by mating or selfing.

Metalaxyl has been reported to induce single mating isolates of *P. infestans* to form oospores after exposure to the chemical (Trout and Ristaino, 1999). The production of oospores in *P. infestans* cultures from major potato growing districts of Uganda could be as a result of continuous use of the fungicide by potato farmers. While it is generally agreed that intense use of pesticides commonly result in development of pesticide resistance (Matthews, 1984), other studies also show that use of very low dosage eventually select resistant strains of the pest (Busvine, 1980). Since there is relatively low usage of pesticides in Uganda agriculture, it is likely that the resistance to metalaxyl may have developed as a result of usage of low dosage of metalaxyl.

4.4.5 Virulence of *P. infestans*

All *P. infestans* isolates examined in this study were pathogenic on potato, but only 44.2% were pathogenic on tomato leaves. Furthermore, *P. infestans* isolates caused more disease (percent leaf area affected) on potato leaves than tomato. This finding clearly reveals that the *P. infestans* isolates from potato were more aggressive on potato than tomato. Legard *et al.* (1995) also reported similar findings. However, it is not known whether *P. infestans* from tomato are able to infect potato in the Ugandan situation, because there was no success in maintaining the tomato isolates for this test. Additional studies are, therefore, required to investigate whether isolates pathogenic to tomato can be virulent on potato. In the USA, there are isolates which attack both tomato and potato (Goodwin *et al.*, 1994; Goodwin *et al.*, 1995).

The results from these experiments provide some information essential for development of effective disease control strategies. The presence of *P. infestans*, isolates virulent on both potato and tomato requires that perhaps similar late blight management strategies be adopted for these crops. This is supported by the fact that that over fifty percent of *P. infestans* isolates that caused disease on tomato leaves were highly resistant to metalaxyl, and the same isolates were able to produce oospores in mating and selfing. Thus, care must be taken to minimise use of metalaxyl (ridomil) on both crops.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The survey in eastern and southwestern Uganda and the experiments set at Namulonge Agricultural Research Institute laboratory were to establish the occurrence of late blight disease in major potato growing areas of Uganda, assess yield loss caused by late blight. This study was also designed to characterise *P. infestans* populations with respect to occurrence of A2 mating type, pathogen resistance to metalaxyl and their virulence on potato and tomato hosts. The surveys revealed that late blight disease was common and severe in all areas studied. The variation in disease levels among the four districts (Kisoro, Kabale, Mbarara, and Mbale) was associated with differences in weather conditions (Callbeck, 1960) and the cultivars grown. However, differences in occurrence of the different pathotypes seemed to be associated with the type of fungicide in use.

The study also indicated that disease levels varied significantly among the potato varieties. Some varieties like Cruza, Musitamya and Rutuku recorded low disease severity, while others such as Victoria, Sangema and Kabale recorded high disease severities i.e., >40% (see chapter 3). The average yields obtained from potato fields in Kabale and Mbarara were only 10.9 t/ha, which is below the average of 15 t/ha for developing countries (FAO/CIP, 1995) and 26 t/ha at Kalengyere Research Station (Hakiza *et al.*, 1997b). Also, the correlation between late blight severity and yield of potatoes was weak ($r=-0.065$, $P=0.71$), possibly due to the fact that there was low

levels of late blight during the survey as a result of the dry spell. This is supported by the work of Adipala (1999) who reported total crop failure due to late blight in parts of Mbale and Kapchorwa in eastern Uganda. Adipala (1999) also reported lower yield losses in southwestern highlands (Kabale and Kisoro) due to widespread use of late blight resistant varieties, such as Rutuku.

This study also attempted to characterise the *P. infestans* population in Uganda. High levels of metalaxyl resistant *P. infestans* isolates were evident from most potato growing areas of the country, but more so in Kabale and Kisoro. The existence of metalaxyl resistant isolates is an indication of the possible existence of new *P. infestans* population in this country, which has also been reported elsewhere (Deahl *et al.*, 1995; Goodwin *et al.*, 1996). Most of the metalaxyl resistant isolates were from those districts reporting use of the fungicide ridomil. Nevertheless, metalaxyl resistance was also detected among those isolates from areas with no or very little metalaxyl usage. Thus, the population of *P. infestans* in Uganda appear more complex than earlier thought, and this might be contributing to the increased occurrence of late blight in the country.

The results also show that *P. infestans* isolates were able to produce oospores with the Al tester isolate and in selfing. This shows the potential for hybridisation and selfing of *P. infestans* in potato growing areas of Uganda. Hybridisation and selfing in *P. infestans* were only known to occur in Mexico (Tooley *et al.*, 1985), but today, the mechanisms have been reported in other parts of the world, such as in Europe (Hohl & Iselin, 1984; Tantijs *et al.*, 1986; Therrien *et al.* 1989), Egypt (Shaw *et al.*, 1985) and Japan (Mosa *et al.*, 1989). Thus, new populations of *P. infestans* may have emerged in Uganda.

However, it was not possible to ascertain whether these are new A1s or A2s. Thus, it was not possible to accept or reject the second hypothesis that A2 mating type occurs in Uganda and has contributed to fungicide resistance. Further studies are needed in this area.

The virulence test showed that, 44.2% of the tested *P. infestans* isolates from potato were able to infect tomato leaves under laboratory conditions. The greatest number of such isolates were highly resistant to metalaxyl and formed oospores. This implies that *P. infestans* isolates from potato have the ability to cause disease to tomato in areas where potato and tomato are grown in the same areas. It is, however, not known if *P. infestans* from tomato have similar ability to cause disease to potato. As such, there is need to conduct similar studies with *P. infestans* from tomato on potato.

The high level of resistance to metalaxyl also suggests that the use of metalaxyl should be planned carefully, as it could increase cost to control late blight using the fungicide (Bradshaw and Vaughan, 1996). Since the commercial product Ridomil contains the contact fungicide Mancozeb in addition to the systemic fungicide metalaxyl, it is recommended to use Ridomil sparingly, and replaceable, at least in part, by Mancozeb alone or by other contact fungicides. Indeed, Kankwatsa (*unpubl.*) has proposed an initial application of ridomil, followed subsequently, by Mancozeb. Additionally, it is important to combine use of fungicides and host resistance. As indicated in chapter 3, varieties such as Rutuku, Cruza and Musityamya showed low levels of late blight attack. Selecting for materials with high levels of horizontal resistance might be the only solution, and this work is already on-going at Kalengyere Research Station (Hakiza *et al.*, 1999).

Based on the results of this study the following areas should be pursued in future studies:

1. Crop loss assessment was conducted for only one season and the results were not conclusive enough to show the relative contribution of the disease to yield loss. Moreover, dry conditions prevailed during the survey, and this is not conducive to late blight development (Henfling, 1987). Therefore, additional assessments should be done with more farmers and for at least three growing seasons.

2. Metalaxyl resistant *P. infestans* isolates were present in late blight samples collected from major potato growing areas of Uganda. There was also evidence of oospore production in mating and selfing of most of the *P. infestans* isolates. These point to the possible existence of A2 mating type in Uganda. However, metalaxyl resistance alone is not sufficient evidence for the existence of A2 mating type because there is no genetic correlation between metalaxyl resistance and mating type (Gisi and Cohen, 1996). The production of oospores was also erratic. It was therefore not known for certain whether the oospores were as a result of mating with the A1 tester or simply due to selfing of the pathogen. In order to confirm the existence of A2 mating type, and more so the possibility that new *P. infestans* population have developed in Uganda, there is a need to test the isolates with A2 tester isolate, and carry-out isozyme and DNA fingerprinting.

3. The *P. infestans* isolates were able to infect potato (variety, Victoria). It is recommended that further collection of the fungus be made from similar locations that

showed different sensitivity to metalaxyl, following which pathogenicity tests be conducted on the different potato genotypes, as this may have epidemiological implications.

There should be renewed attempts to isolate *P. infestans* from tomato and conduct virulence test on potato and tomato to confirm the possibility of having *P. infestans* biotypes attacking both potato and tomato.

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APPENDIX

Appendix 1: Mean rainfall and temperature in Kabale, Mbarara and Mbale districts of Uganda, 1997-1999.

KABALE DISTRICT

Kabale Meteorology Station

	Season 1				Season 2			
	March	April	May	June	Sept.	Oct.	Nov.	Dec.
1997								
Rainfall (mm)	113.5	122.0	148.8	32.8	24.8	155.1	195.8	150.8
Maximum temperature C	24.3	23.2	23.3	23.8	26.0	26.6	23.0	23.1
Minimum temperature C	11.8	12.9	12.3	10.6	10.6	12.7	13.3	13.0
1998								
Rainfall (mm)	101.2	171.3	169.6	18.5	87.4	153.9	58.4	79.3
Maximum temperature C	25.3	24.3	25.4	24.6	25.2	24.5	24.3	25.4
Minimum temperature C	13.0	13.6	12.1	10.5	10.6	11.1	12.1	10.4
1999								
Rainfall (mm)	144.9	71.6	51.0	Trace	-	-	-	-
Maximum temperature C	23.6	23.5	23.1	24.5	-	-	-	-
Minimum temperature C	11.9	11.8	10.9	9.8	-	-	-	-

Source: Meteorology Department, Kampala

b) Kalengyere Station

	Season 1				Season 2			
	March	April	May	June	Sept.	Oct.	Nov.	Dec.
1997								
Rainfall (mm)	114.4	131.2	85.5	24.9	50.2	75.5	145.4	144.0
Maximum temperature C	20.5	19.8	21.2	21.6	24.0	20.4	19.9	21.2
Minimum temperature C	11.2	10.6	11.1	11.2	11.7	10.8	10.4	10.9
1998								
Rainfall (mm)	124.9	89.1	106.8	57.6	123.9	49.9	99.8	
Maximum temperature C	21.7	21.3	20.9	22.8	21.4	21.3	21.7	20.3
Minimum temperature C	11.3	10.3	10.9	11.8	11.1	11.2	11.4	10.9
1999								
Rainfall (mm)	132.1	58.7	33.9	Nil	-	-	-	-
Maximum temperature C	20.4	20.9	21.5	22.0	-	-	-	-
Minimum temperature C	10.6	10.9	11.4	12.3	-	-	-	-

Source: Kalengyere Research Station

BIARARA DISTRICT

Biarama Station

	Season 1				Season 2			
	March	April	May	June	Sept.	Oct.	Nov.	Dec.
1997								
Rainfall (mm)	69.5	206.8	119.1	25.7	21.3	122.3	76.0	231.0
Maximum temperature C	28.4	25.7	26.3	27.1	29.3	27.1	25.6	25.9
Minimum temperature C	16.1	16.1	15.6	14.8	14.9	16.0	16.3	16.0
1998								
Rainfall (mm)	60.1	88.1	100.3	1.5	Na	Na	Na	Na
Maximum temperature C	28.4	25.7	26.3	27.1	29.3	27.1	25.6	25.9
Minimum temperature C	16.2	16.9	16.8	14.5	Na	15.6	15.3	
1999								
Rainfall (mm)	58.4	74.6	3.7	Nill	--	-	-	-
Maximum temperature C	Na	na	na	Na	-	-	-	-
Minimum temperature C	Na	na	na	Na	-	-	-	-

Source: Meteology Department Kampala na= data not available

Bzegambe Forest Station

	Season 1				Season 2			
	March	April	May	June	Sept.	Oct.	Nov.	Dec.
1997								
Rainfall (mm)	50.4	206.3	202.6	31.1	135.6	111.8	159.5	181.5
Maximum temperature C	*							
Minimum temperature C	*							
1998								
Rainfall (mm)	146.1	94.8	99.1	na	90.7	Na	na	40.4
Maximum temperature C	*							
Minimum temperature C	*							
1999								
Rainfall (mm)	na	na	na	na				
Maximum temperature C	*				-	-	-	-
Minimum temperature C	*				-	-	-	-

Source: Meteology Department Kampala *= no facility to record parameter na=data not available

MALE DISTRICT

Mayanya Station

		Season 1				Season 2			
		March	April	May	June	Sept.	Oct.	Nov.	Dec.
71									
rainfall (mm)	127.0	462.7	168.7	93.4	23.7	369.4	210.2	99.5	
maximum temperature C	na								
minimum temperature C	na								
73									
rainfall (mm)	12.5	172.2	349.4	143.6	262.0	237.7	191.8	18.3	
maximum temperature C	na								
minimum temperature C	na								
79									
rainfall (mm)	na								
maximum temperature C	na					-	-	-	-
minimum temperature C	na					-	-	-	-

Source: Meteorology Department Kampala na= data not available

Appendix 2 Mean squares of colony diameter (mm) of *P. infestans* under the metalaxyl resistance test of the combined analysis of the repeat experiments (Runs) for May and November 1998, and May 1999 collection.

Source	DF	Meas squares
May 1998 collection		
Replications	3	30.1*
Runs	2	237.1**
Isolates	25	2824**
Metalaxyl concentration (MC)	2	169260**
Isolates x MC	50	474.7**
Run x Isolates	50	125.6**
Run x MC	4	384.4**
Run x Isolates x MC	100	82.7**
Error	699	10.6
November 1998 collection		
Replications	3	22.9 ns
Runs	2	42.2*
Isolates	25	2604.7**
Metalaxyl concentration (MC)	2	199180.5**
Isolates x MC	50	569.7**
Run x Isolates	50	89.7**
Run x MC	4	309.2**
Run x Isolates x MC	100	47.2**
Error	699	12.1
May 1999		
Replications	3	6.6.1 ns
Runs	2	2741.6**
Isolates	29	2741.6**
Metalaxyl concentration (MC)	2	143408.0**
Isolates x MC	58	652.4**
Run x Isolates	58	63.8**
Run x MC	4	73.7**
Run x Isolates x MC	116	33.9**
Error	807	14.2

ns denotes not significant, * & ** significant at $P \leq 0.05$ and $P \leq 0.001$ respectively

Appendix 3 Mean squares of transformed lesion length and percent area affected by late blight for the repeat experiments (Runs) for May and November 1998, and May 1999 collection.

Source	DF	Meas squares	
		Lesion length	Percent area
May 1998 collection			
Replications	3	0.9**	76.9**
Runs	2	3.3**	673.9
Isolates	20	14.2**	827.2
Hosts	1	344.3	17773.9
Isolates x Hosts	20	11.3**	566.7**
Run x Isolates	28	1.3**	54.9**
Run x Host	2	0.1 ns	174.4**
Run x Isolates x Host	26	0.7**	105.9**
Error	293	0.2	14.0
November 1998 collection			
Replications	3	0.2 ns	54.5*
Runs	2	2.5**	795.6**
Isolates	17	20.1**	1062.2**
Hosts	1	489.3**	26018.5**
Isolates x Hosts	17	15.9**	736.2**
Run x Isolates	34	0.8**	76.3**
Run x Host	2	0.3 ns	446.4**
Run x Isolates x Host	34	0.7**	48.2**
Error	267	0.1	11.8
May 1999 collection			
Replications	3	0.02 ns	1.7 ns
Runs	2	0.8*	107.7**
Isolates	22	16.1**	695.9**
Hosts	1	307.8**	12135.5**
Isolates x Hosts	22	13.8**	573.5**
Run x Isolates	33	0.6**	64.3**
Run x Host	2	0.4 ns	40.6*
Run x Isolates x Host	33	0.5**	61.8**
Error	345	0.14	11.8

ns denotes not significant, * & ** significant at $P \leq 0.05$ and $P \leq 0.001$ respectively
 Lesion length underwent square root transformation ($x+0.5$), % area affected arcsine transformation ($x+1$)

Appendix 4 Categories of metalaxyl sensitivity of *Phytophthora infestans* isolates collected in 1998 -1999.

Highly metalaxyl resistant isolates: growth on media with 5µg/ml and 100µg/m metalaxyl ≥ 40% of 0µg/ml;

2002 M	4002 M	4009 M	2038 M
2001 MS	4006 MS	2026 MS	2039 MS
2002 MS	2009 MS	2028 NO	2040 M
2001 M	2021 S	2031 M	2042 S
2008 MS	2022 MS	2033 NO	2046 MS
2009 MS	3015 MS	2034 MS	3021 MS
2010 M	3017 MS	2035 M	4010 M
2012 MS	3020 MS	2036 MS	4011 MS
2001 MS	4008 M	2037 M	4012 MS

Isolate source: 1000+=Kisoro; 2000+= Kabale; 3000+= Mbarara; 4000+=Mbale
Oospore production: M=oospores in mating only; S=oospores in selfing only;
MS=oospores in both mating & selfing; NO=no oospores in mating and selfing

Intermediate isolates: growth on media with 5µg/ml metalaxyl ≥ 40% of 0µg/ml

2003 MS	4003 MS	2016 MS	2025 NO
2004 NO	4005 MS	2017 MS	2032 NO
2005 MS	1004 MS	3016 MS	4013 MS
2006 M	1007 MS	4007 MS	4014*
2013 MS	2005 M	1009 S	

Isolate source: 1000+=Kisoro; 2000+= Kabale; 3000+= Mbarara; 4000+=Mbale
Oospore production: M=oospores in mating only; S=oospores in selfing only;
MS=oospores in both mating & selfing; NO=no oospores in mating and selfing
Isolate not tested for mating type

Sensitive isolates: growth on media with 5µg/ml and 100µg/m metalaxyl ≤ 40% of 0µg/ml.

2001 NO	1005 M	2015 NO	2027 MS
2003 MS	2004 S	2018 M	2029 S
2003 MS	2006 NO	2023 NO	2030 MS
2002 MS	2008 NO	3014 MS	2043 M
2007 NO	2010 MS	1008 S	2045 S
2011 NO	2013 NO	2024 S	3022 M
2004 MS	2014 MS		

Isolate source: 1000+=Kisoro; 2000+= Kabale; 3000+= Mbarara; 4000+=Mbale
Oospore production: M=oospores in mating only; S=oospores in selfing only;
MS=oospores in both mating & selfing; NO=no oospores in mating and selfing

Appendix 5 Site description of KISORO isolates (Isolate No 1000+)

Isolate No	Sample no Coll. date	Place	Geograph. location GPS	Cultivar LB Score %
1001	12a 22-5-98	Nyakinama	S 01°17.802' 1832 masl E 029°36.632	Victoria 3 sprays Small plot 10
1002	13a 22-5-98	Nyakinama	S 01°17.802' 1832 masl E 029°36.632'	Victoria 2 sprays larger plot 10
1003	14a 22-5-98	Nyakitembe	S 01°15.231' 2062 masl E 029°46.984'	Cruza-148 Small plot 50
1004	12b 10-11-98	Nyarusiza	S 01°18.877' 1974 masl E 029°40'	Sutama Small plot 10
1005	21b 10-11-98	Butengo	S 01°11.147' 2278 masl E 029°46.792'	Rutuku 2 sprays large plot 15
1007	22b 11-11-98	Rwamashenyi	S 01°586 2371 masl E029°46.045	Rutuku 1 spray Larger plot 15
1008	22c 27-5-99	Kifumba	-	Rutuku 2 sprays large plot 10
1009	23c 27-5-99	Mukateretere	-	Rutuku 1 spray large plot

Rutuku = CIP 720097; Victoria = CIP 381381.20; Cruza148 = CIP 720118

Small plot = 1 to few 100m², larger = around 0.2-0.5 acres, large = 1 acre or more

Appendix 6 Site description of KABALE isolates (Isolate No 2000+)

Isolate No	Sample No Coll. date	Place	Georgraph. Location GPS	Cultivar %	LB score
2001	22a 22-5-98	Rusekye Near KRS larger	S 01°13.214' 2352 masl E029°48.520'	Victoria 2 sprays D	50
2002	23a 22-5-98	Murukoro	S01°12.192' 2160 masl E029°48.420'	Victoria 3 sprays D/R largre	10
2003	28a 23-5-98	Bukinda (Mrs Kisiizi) larger	S01°11.085' 1855 masl E 030°06.280'	Rutuku 2 sprays D	10
2004	16b 10-11-98	K.R.S	S 01°13.229' 2525 masl E 020°47.769	Victoria large 1 spray D	45
2005	17b 10-11-98	K.R.S	-do-	Victoria 1 spray Large plot	25
2006	17b 10-11-98	KRS	-do-	-do-	
2008	17b 10-11-98	KRS	-do-	-do-	
2009	20b 11-11-98	Ntungamo Near K.R.S		Rutuku 1 spray D Larger plot	10
2010	20b 11-11-98		-do-	-do-	
2013	25b 11-11-98	Haisesero		Rutuku 2 sprays Larger plot	40
2014	26b 11-11-98	Hamurwa		Victoria No spray Small plot	65
2015	26b 11-11-98	-do-		-do-	
2016	26b 11-11-98	-do-			
2017	26b 11-11-98	do		do	
2018	27b	Kagano		Victoria	70

	11-11-98		2 sprays D larger plot	
021	30b 11-11-98	Kibuzige	Sengema No spray Small plot	45
022	31b 11-11-98	Kachwekano	Victoria 2 sprays Large plot	25
023	29b 11-11-98	Bukinda	Victoria Larger plot	20
024	24c 28-5-99	K.R.S	Victoria 1 spray D larger plot	25
025	24c 28-5-99	-do-		
026	24c 28-5-99	K.R.S	Victoria	
027	25c 28-5-99	K.R.S	Victoria 1 spray large plot	30
028	25c 28-5-99	K.R.S	-do-	
029	26c 28-5-99	Ntungamo	Rutuku no spray Larger plot	30
030	26c 28-5-99	-do-	-do-	
031	27c 28-5-99	Katojo	Victoria 1 spray	40
032	27c 28-5-99	-do-	larger plot	
033	27c 28-5-99	-do-	-do-	
034	27c 28-5-99	-do-	-do-	
035	28c 28-5-99	Murukoro	Victoria 1 spray large plot	45
036	28c 28-5-99	-do-	-do-	
037	28c	-do	-do-	

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	28-5-99			
3	29c	Haisesero	Rutuku	25
	28-5-99		1 spray	
9	31c	Mubwindi	large plot	
	28-5-99		Victoria	50
			Small plot	
10	31c	-do-	-do-	
	28-5-99			
12	32c	Hamurwa	Cruza	25
	28-5-99		Small plot	
13	32c		-do-	
	28-5-99			
15	32c	-do-		
	28-5-99			
16	32c	Bubare	Victoria	40
	28-5-99		larger plot	

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Rutuku = CIP 720097; Victoria = CIP 381381.20; Cruza148 = CIP 720118

Small plot = 1 to few 100m², larger = around 0.2-0.5 acres, large = 1 acre or more

IRS = Kalengyere Research Station D = Dithane M-45 R = Ridomil MZ

Appendix 7 Site description of MBARARA isolates (Isolate No 3000+)

Isolate No	Sample No	Place	Geograph. Location	Cultivar	LB
	Coll. date		GPS		%
001	34a 29-5-98	Rukandagi	S 00°42.34' 1590 masl E 030°32.121'	Victoria 2 sprays larger plot	5
002	-do-	-do-		do-	
003	-do-	-do-		-do-	
004	35a 29-5-98	Karabo	S 00°47.42' 1850 masl E 030°37.311'	Victoria larger plot	10
005	-do-	-do-		-do-	
006	39a 29-5-98	Ngoma	S 00°45.14' 1845 masl E 030°36.210'	Victoria 2 sprays large plot	10
007	-do-	-do-		-do-	
008	40a 29-5-98	Rukarabo	S 00°46.862' 1850 masl E 030°37.312'	Victoria large plot	10
009	-do-		-do-	-do-	
011	-do-		-do-	do-	
012	-do-		-do-	-do-	
013	-do-		-do-	-do-	
014	37b 16-11-98	Rukarabo		Victoria larger plot	10
015	38b 16-11-98	Nyakibale		Victoria Large plot	10
016	40b 16-11-98	Bugamba		Victoria large plot	15
017	41b 16-11-98	Mwizi TC		Victoria larger plor	15
020	43b 16-11-98	Mbarara MC G. Turyamureeba		Tomato + potato in Bananas	45
021	4c 15-5-99	Karamurani Mwizi		Victoria Large	25
022	5c 15-5-99	Bugamba		Victoria Large	10

Appendix 8 Site description of MBALE isolates (Isolate No 4000+)

Isolate No	Sample No Coll. date	Place	Georgraph. Location GPS %	Cultivar	LB score
001	45a 5-6-98	Bugaheme	N01°03.400' 1843 masl E 034°14.550'	Maboni 3 sprays large	15
002	47a 5-6-98	Bunadutu	N01°03.106' 1963 masl E 034°14.289'	Maboni Large plot	25
003	48a 5-6-98	Busuyo	N01°03.095' 1952 masl E 034°14.594'		35
004	52a 5-5-98	Bubirambi	N01°01.980' 2012 masl E 034°14.141'	Maboni 2 sprays larger plot	20
005	54a 5-6-98	Naburwa	N01°02.111' 1978 masl E 034°14.103'	Wafrika large	45
006	58a	Budware/ Rwasohe	N01°03.723' 1830 masl E 034°14.766'	Maboni	30
007	52b 4-12-98	Busuyo large		Maboni	20
008	53b 4-12-98	Bumbase		Maboni large	
010	17c 21-5-99	Lwangasa		Maboni large	15
011	-do-	-do-		-do-	-do-
012	19c	Bunabulo upper		Maboni	25
013	-do-	-do-		-do-	-do-
014	21c	Rusohe		Maboni	15

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Appendix 9 CIP Field Key for Assessment of Potato Late Blight

P scale Time	Blight		Symptoms
	Mean	% limits	
	0		No late blight observable
	2.5	traces to < 5	Late blight present. Maximum 10 lesions per plant
	0	5 to <10	Plants look healthy, but lesions easily seen at closer distance. Maximum foliage area affected by lesion or destroyed correspond to no more than 20 leaflets.
	25	15 to < 35	Late blight easily seen on most plants. About 25% of foliage is covered with lesion or destroyed.
5	50	35 to < 65	Plot looks green; however, all plants are affected. Lower leaves are dead. About half the foliage area is destroyed.
6	75	65 to < 85	85Plot looks green with brown flecks. About 75% of each plant is affected. Leaves of the lower half of the plants are destroyed.
7	90	85 to <95	Plot neither predominantly green nor brown. Only top leaves are green. Many stems have large lesions.
8	97.5	95 to < 100	Plot is brown-coloured. A few top leaves are still have some green areas. Most stems have lesions or dead.
9	100		All leaves and stems dead.

The description of symptoms is based on plants with 4 stems and 10 to 12 leaves per stem
Source: Henfling, 1987.

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