CHARACTERISTICS OF *Phytophthora infestans (Mont.) de Bary* ISOLATES COLLECTED FROM POTATO IN CANADA DURING 1993 AND 1994

by

Cathy I. Chycoski

B.Sc.Agric., University of Saskatchewan, 1985

B.A., University of Saskatchewan, 1979

M.P.M.THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

Master of Pest Management

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

December, 1995

APPROVAL

Name: **Cathy Irene Chycoski**

Degree: **Master of Pest Management**

Title of Thesis:

CHARACTERISTICS OF *PHYTOPHTHORA INFESTANS (MONT.) DE BARY* ISOLATES COLLECTED FROM POTATO IN CANADA DURING 1993 AND 1994.

Examining Committee:

Chair: Dr. S. Lee, Assistant Professor

Dr. Z. K. Punja, Assoc. Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. J. Rahe, Professor,
Department of Biological Sciences, SFU

Mr. David Ormrod, Plant Pathologist
B.C. Ministry of Agriculture, Fisheries and Food
Public Examiner

Date Approved December 14, 1995

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

Characteristics of Phytophthora infestans (Mont.) de Bary isolates collected from potato in Canada during 1993 and 1994.

Author:

(signature)

Cathy Chycoski

(name)

December 14, 1995

(date)

ABSTRACT

The incidence and distribution of A1 and A2 mating types of *P. infestans* in Canada and of metalaxyl sensitive (MS) and insensitive (MI) strains were monitored during 1993 and 1994. Diseased leaves from about 1,200 plants were collected from 26 cultivars in 138 potato fields from five provinces at various times (June - September) during the growing season. The most extensive sampling (80% of total samples) was conducted in British Columbia (B.C.). About 400 isolates of *P. infestans* were characterized for mating type and/or metalaxyl sensitivity from a total of 775 isolations. In 1993, the A1 mating type was found in all provinces, while A2 occurred only in B.C. and was distributed throughout the entire province. In 1994, the A2 mating type was also found in New Brunswick (N.B.). Twenty-three fields sampled in B.C. in 1993 had both A1 and A2 mating types in the same field on different plants. In N. B. in 1994, both A1 and A2 were isolated from the same leaf samples collected from 19 different fields. Examination of cleared leaves in the microscope revealed the presence of oospores, illustrating the potential for oospore production in naturally infected leaves when both mating types of *P. infestans* are present. Metalaxyl sensitivity tests based on growth of isolates at 0 and 50 μg/ml of metalaxyl showed that all isolates collected from provinces other than B.C. in 1993 were MS. Isolates from B.C. showed a wide range of growth rates in the absence and presence of metalaxyl, and 79% of the isolates were MI. There was no apparent correlation between the recovery of MI strains and whether metalaxyl had been applied to the field during the same growing season. Both MS and MI strains were recovered from the same sample in six fields. A low frequency of A1 MS isolates was always recovered in B.C. A high proportion of isolates collected early in the season (June) from B.C. over two years were A2 and MI. MI isolates were

recovered at a higher or equal frequency than MS isolates toward the end of the season (September) in B.C. and N.B. Isolates of A2 mating type were found in a higher proportion than A1 at the end of both seasons in B.C. and at the end of the 1994 season in N.B. The mean growth rates on agar of isolates grouped into A1 and A2 categories were significantly different in B.C. and N.B. in 1994 with the A2 mating type growing faster than the A1. However, isolates grouped in MS and MI categories were significantly different from each other only in the 1993 B.C. isolates, when the MI isolates grew faster than MS. The results from this study illustrate the dynamic nature of populations of *P. infestans* in Canada, both within and between growing seasons, and demonstrate some variation in the population in B.C.

Dedication

To the excellent teachers and extension agrologists I have had the privilege of working with over the years, especially Mr. Don Dabbs, University of Saskatchewan, Mr. David Ormrod, British Columbia Ministry of Agriculture, Fisheries and Food, and Mr. Paul Froese, Agriculture Canada.

Acknowledgments

Funding for this research was provided by grants from Ciba Canada, the Natural Sciences and Engineering Research Council of Canada, the B.C. Vegetable Marketing Commission and the B.C. Ministry of Agriculture, Fisheries and Food.

Thank you to Pam Morris, Heidi Zimmerling, Faizel, Baldeep Bains, Tei Kyung and Jim Burton who provided excellent and creative technical assistance. Thank you to all of the numerous individuals and extension personnel who sent in late blight-infected potato samples or fungal cultures for this study.

Thank you to all the farmers in British Columbia, Alberta, and New Brunswick who were gracious enough to allow the collection of diseased leaves from their fields. Thanks go to Dr. Z. K. Punja and Dr. J. Rahe for participating in the committee guiding this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER 1

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is an important disease of potato worldwide (Fry et al 1993). The disease can decrease yield and lower tuber quality, size and dry matter content, resulting in significant crop losses during the growing season and also in storage. Tubers that are infected in the field rot rapidly in storage. If epidemics are not controlled, 100% crop loss is not uncommon (Stevenson, 1993). Potato late blight was unknown in Europe until the 1840's when it caused in devastating crop losses including the Irish potato famine (Nelson, 1995). The pathogen was probably introduced to Europe on seed or table potatoes from Mexico, which is considered the probable center of origin of potato late blight (Niederhauser,1989). Recent anecdotal evidence, however, suggests that *P. infestans* may have originated in the Andean mountains of Peru (Abad et al, 1995).

Disease symptoms

Late blight symptoms on potato plants first appear as pale green, circular or irregular shaped, water soaked lesions, which rapidly turn brown. The border of the lesion often appears yellowish-green; on the lower side of the leaf, the lesion is surrounded with a ring of sporangiophores, giving the appearance of a white mildew. Late blight lesions develop on leaves, stems, and petioles, with the symptoms in the latter developing in the leaf axil where moisture accumulates. At later stages of an epidemic, several lesions can coalesce on a single leaflet (Van der Plank, 1968). If the weather becomes hot and dry, the lesions turn brown and dry up. Tubers are infected during the growing season

by sporangia that are washed from the stem and leaves of the plant and through the soil, or during harvest if freshly dug tubers contact sporulating lesions. The external symptom on the tuber is sunken epidermal tissue, which remains brown and dry until secondary infection occurs. Just below the epidermis, the tissue is brown and grainy but not slimy. Infected tissue is readily colonized by secondary pathogens, such as *Fusarium* and *Pythium* species, and soft rot bacteria (*Erwinia* species) (Parry,1990).

Potato late blight has direct and indirect effects on crop yield due to the accelerated leaf loss during the growing season, particularly if the disease occurs early in the season (Van Oijen, 1992). Crop yield is directly affected by expanding lesions which reduce leaf area and photosynthetic area. However, the presence of the fungus in the foliage of potatoes will increase the rate of senescence in uninfected parts of the haulm, the aerial stems and leaves of the plant, thus contributing to loss of photosynthetic area (Van Oijen, 1992). Reduced photosynthetic area has a direct effect on tuber quality by lowering the dry matter content in the tuber (Dowley and O'Sullivan, 1995).

Isolation of the pathogen

The pathogen can be isolated by a number of methods using selective media or potato tissue Fragments of diseased tissue, which may or may not be surface sterilized, are placed on selective agar medium containing rye grain extract, or 10% V - 8 juice, and penicillin G, polymoxin, and pimaricin, (Deahl et al, 1991, Fyfe and Shaw, 1992). A piece of the agar containing mycelium from the edge of the colony initiated from the diseased tissue is transferred to fresh media to establish a pure culture (Fyfe and Shaw, 1992). Isolation has also been made by placing diseased tissue below a one centimeter thick slice of potato tuber and incubating at 15°C until sporulation can be seen on the surface

of the tuber slice (Davidse et al, 1981). The fungus is then transferred on a weekly basis to fresh potato tuber tissue.

Characteristics of the pathogen

P. infestans belongs to the class *Oomycetes* due to the presence of cellulose in cell walls and the motile zoospores, the order *Peronosporales* due to the shape of the sporangia, and the family *Pythiaceae* due to the indeterminate growth habit of the sporangiaphores (Carlile and Watkinson, 1994, Weier et al, 1982). *P. infestans* is heterothallic and bisexual, with two mating types designated as A1 and A2 (Galindo and Gallegly, 1960). Although it is bisexual, selfing rarely occurs (Galindo and Gallegly, 1960, Shattock et al, 1986). It is considered a diploid organism (Shattock et al, 1986, Shaw, 1987) and an obligate parasite. The mycelium is coenocytic, multinucleate and aseptate, although cross walls do form in old cultures *in vitro*. Sporangia are borne singly on the branch tips of the alternately branched sporangiophores. The sporangium is opaque, white and lemoniform, with a papilla at the distal end. Sporangium are released from the sporangiaphore by a twisting and popping of the sporangiophore and are carried on air currents. Zoospore formation within the sporangia is temperature dependent. Vegetative spores and mycelium can exist and remain infectious in plant debris for at least one week (Drenth et al, 1994a).

Growth and infection

Development of propagation structures and germination occurs over a wide range of temperatures in this pathogen. Sporangia develop between 2.8 and 26°C, but the optimum temperatures are between 17 and 22°C. Six to eight zoospores form in the sporangia when the temperature drops below 18°C, but

the optimum temperature for zoospore formation is 12 to 15°C (Dowley and O'Sullivan, 1995). Zoospores are released from the sporangium at 8 to 13°C, via the papilla located at the distal end of the sporangium (Dowley and O'Sullivan, 1995). The bi-flagellate zoospores move along a water film on the host tissue prior to encystment (Dowley and O'Sullivan, 1995). Direct germination of the sporangia occurs between 21°C and 26°C at 90% relative humidity. Germination of both zoospores and sporangia requires a water film. However, too large a water droplet can inhibit penetration of tissue due to inability of germ tubes to reach the plant surface (Lapwood, 1961). The germ tube penetrates the host by forming an infection peg, and enters an epidermal cell directly or through a stoma (Gees and Hohl, 1988). Infection peg penetration of plant tissue is followed by haustorium growth into surrounding cells (Dowley and O'Sullivan, 1995). Once in the host, the optimum temperature for fungal growth is between 18 to 21°C (Vartanian et al 1985). Sporangiophores may emerge though the stomata in as little as five days under optimum conditions (Dowley et al 1995). Temperatures above 30°C are unfavorable for growth but *P. infestans* can survive (Vartanian et al 1985).

Disease development and spread

Diseased tissue increases exponentially over a growing season during a late blight epidemic (Van der Plank, 1975). Spores are spread short distances by water splash and over longer distances by wind, farm equipment and diseased tubers (Stevenson, 1993). The general pattern for pathogen dispersal is size expansion of small disease foci throughout the season. Early in the season, small disease focal points originating from cull piles or volunteer potato acting as over wintering hosts to the pathogen develop at short distances from the original disease focus. The disease foci generally expand in size throughout

the season. Windy, wet weather can transport spores over long distances with recorded spore flight ranges of over 60 kilometers initiating new disease foci (Van der Plank, 1975). Farm equipment such as sprayers can distribute spores, and disease can often be seen developing along tractor rows (Van der Plank, 1975). .

Potato production in British Columbia

Solanum tuberosum (L.), potato, is believed to have originated from *Solanum* species native to Peru (Abad et al 1995). The potato that was first imported to Europe was an Inca food staple, *Solanum andigena* (National Research Council, 1989). Present day cultivars have been developed from *S. andigena* and *S. demissum.*

Potatoes are cultivated for seed production, table consumption and for processing in British Columbia (B.C.). The Pemberton Valley produces exclusively tissue culture derived seed potatoes. Potatoes are a quarantined crop in this valley, making it possible to produce seed potatoes free from bacterial ring rot and virus diseases. Seed potatoes are also grown on Vancouver Island, the lower mainland, and in the Okanagan Valley. Table and processing potatoes are grown in the areas outside Pemberton, and in the central plateau region of B. C. Potato cultivars are chosen for maturation date: early-, mid- or late-season crops, as well as skin and flesh colour (B.C.M.A.F.F.,1993). Entire fields of early season cultivars grown for spring harvest as new potatoes (small, tender, fresh-market potatoes) are often covered with a floating porous mulch to increase the temperature around the plants to result in an earlier harvest date and thus a premium price for the crop. Mid- and late- season cultivars are hilled before the rows close in to ensure higher yield

and quality by adequate soil coverage of the tubers. Overhead irrigation is applied as necessary to ensure good tuber size. Pesticides are usually applied upon recommendation of crop monitors or on a calendar basis. When the crop is mature, the vines are top killed to prevent further tuber growth by applying Reglone (diquat) or by flailing the haulm (B.C.M.A.F.F.,1993). Tubers are placed in temperature controlled storage at 10°C after harvest to suberize them, and then the temperature is lowered for long term storage (B.C.M.A.F.F.,1993).

Insect and disease pests of potato

The major insect pests in B.C. are aphids (for seed production), tuber flea beetle, wireworm, and Colorado potato beetle (excluding the coastal region of B. C. and the Pemberton Valley). The important diseases and pathogens are aster yellows, *Corynebacterium sepedonicum* (bacterial ring rot), *Rhizoctonia solani* (black scurf), *Erwinia carotovor*a subsp. *atroseptica* (blackleg) *Alternaria solani* (early blight), *Phytophthora infestans*, leaf roll virus, PVX and PVY, (potato mosaic virus), root knot nematode, *Streptomyces scabies* (scab), *Helminthosporium solani* (silver scurf) and *Verticillium albo-atrum* (verticillium wilt) (B.C.M.A.F.F.,1993). Several of these diseases can be avoided or reduced by planting certified seed potatoes.

Host resistance to late blight

Potato cultivars with resistance to late blight have been developed since 1900 through breeding and selection for disease avoidance and tolerance. Earliness has been used as a strategy to escape disease loss, since late blight typically does not reach epidemic proportions until the end of the growing season. However, early cultivars do not always escape disease since a cool,

wet spring can precipitate an "early" late blight epidemic. Disease avoidance is correlated to the distribution of tubers in relation to the central stem as varieties with longer stolons and tubers growing deeper and more widely spaced have a lower chance of being infected by sporangia washed through the soil than are varieties with tubers clustered near the soil surface (Russell, 1978).

Breeding programs have introduced cultivars with varying degrees of resistance to *P. infestans*. Foliar and tuber resistance is often linked (Russsel, 1978). Cultivars with some late blight resistance are Krantz, Belchip, Desiree, Eramosa, Fundy, Lemhi Russet, Kennebec, Nooksack, Nipigon, Onaway, Ontario, Sebago, Shepody and Rosa. Some susceptible cultivars are Atlantic, BelRus, Irish Cobbler, Katahdin, LaRouge, Monona, Norchip, Norgold Russet, Norland, Russet Burbank, Sangre, Superior, Viking and White Rose (Van der Plank, 1975, Platt and McRae, 1990, Stevenson, 1993, BCMAFF, 1995). Cultivar resistance varies among regions as seen with the cultivar Belchip which is designated as susceptible by Stevenson (1993) and having some resistance by Platt and McRae (1990). Considerations such as market acceptance, shape and color play a greater role than disease resistance in cultivar choice for commercial production.

There are two main types of resistance against late blight in potatoes: specific (monogenic or vertical) and polygenic (horizontal). A cultivar that is more resistant to some races of pathogens than others exhibits mainly vertical resistance, which is characterized by the system of R-gene resistant cultivars. Plant breeders have used wild *Solanum* species from Mexico as the primary sources of specific resistance genes (R - genes) which contribute to vertical disease resistance. R - genes first were introduced by hybridizing *Solanum tuberosum* subsp. *tuberosum* L. with *S. demissum*. The resistance mechanism is a hypersensitive reaction involving rapid death of cells invaded by the

pathogen. Plant production of the phytoalexins lubimin and chlorogenic acid and enzymes such as phenylalanine ammonia lyase is increased in response to infection by different races of the pathogen (Russell, 1978).

Horizontal resistance, however, is generally evenly spread against all races of the pathogen (Van der Plank, 1968). Non-race-specific horizontal resistance, controlled by multiple genes, may provide less ephemeral resistance than R - gene specific resistance because it incorporates different levels and types of resistance. The different horizontal resistance characteristics found in potatoes are 1) resistance to infection, 2) inhibition of mycelial growth in host tissues, 3) increased incubation period in the host and 4) reduced or delayed sporulation of the pathogen (Russell, 1978). Some clones of *S. tuberosum* spp. *andigena* have shown high levels of horizontal resistance and contain no R-genes (Russell, 1978). Andigena is an important food crop in the Andes (National Research Council, 1989). *S. goniocalyx* and *S. phureja* have some resistance to *P. infestans* but are important food crops only within areas of South America where they have been cultivated traditionally (National Research Council, 1989).

Pathogen virulence

As new R - genes have been incorporated into cultivated potatoes, R-gene specific, complex pathogenic races of *P. infestans* capable of overcoming specific resistance genes or combinations of the genes in the potato host have been identified by using differential hosts (Gallegly, 1968). Virulence is the ability of a race of pathogen to overcome specific resistance genes in a host plant. There are eleven known virulence factors, r1 - r11, which are observed in various combinations within complex races of *P. infestans* (Drenth et al, 1994b). The races have become widely distributed, often without the selection pressure

of host resistance, and many of these races are virulent against a wide range of host resistance genes (Russell, 1978).

There are several theories to explain the rapid development of pathogenic races: mutation, adaptive parasitism and sexual reproduction. Mutation is the accepted explanation for the mechanism which gives rise to new race characteristics. Adaptive parasitism may occur because of slow fungal growth on senescent leaves of resistant potato cultivars. If the fungus is able to reproduce with subsequent generations living on the host, each generation of the pathogen may exhibit incrementally increased virulence against the formerly resistant host (Black, 1952). The mycelium of *Phytophthora* is diploid; thus, heterokaryon production is a potential source of variation. Gallegly (1968) observed that since the fungus was able to grow slowly on senescent leaves of resistant varieties, mitotic crossing over could occur with resistant tissue acting as a selective medium. Somatic hybridization has been observed in *P. infestans* (Shaw and Shattock, 1991). Sexual reproduction could increase diversity of virulent races. Recombination of virulent races has been observed in offspring produced as a result of sexual reproduction of *P. infestans* (Gallegly, 1968). Virulence tests of the two mating types in terms of R-gene differentials showed two A2 mating types collected from Pennsylvania, USA and B.C., Canada had the same virulence as eight A1 isolates (Deahl et al, 1991). The A1 mating type has a wide range of virulence (Gallegly, 1968). Further study of virulence of isolates belonging to the different clonal lineages based on mating type, allozyme and DNA fingerprint data, has shown that variation in virulence follows clonal lines, with little variation in virulence within clones (Goodwin et al, 1995).

Mating types

P. infestans isolates are heterothallic with two mating or compatibility types, designated as A1 and A2. Both types are morphologically identical. Isolates of each mating type are capable of forming antheridia and oogonia (Galindo and Gallegly, 1960). In Central Mexico, which is considered to be a possible center of origin of *P. infestans*, A1 and A2 occur at a ratio of approximately 1:1 (Niederhauser, 1989). Gametangia are formed by an isolate in the presence of an isolate of compatible mating type (Galindo and Gallegly, 1960). Oospores are rarely formed by single spore isolates.

Gametangia formation occurs when hyphae of compatible mating types grow towards one another (Courtice and Ingram, 1987). The oogonial hypha penetrates the antheridial hypha with an appressorium. Cross wall formation then forms a stalk in the antheridial hypha, preventing further movement of nuclear material into the antheridium. The oogonial hypha then emerges from the antheridium, thus the antheridium is amphygynous as it surrounds the oogonium. Protoplasm flows into the swelling oogonial hypha until a cross wall is laid down in the oogonial stalk within the antheridium. A fertilization tube then extends from the antheridium into the oogonium. A thick double wall forms around the oospore after fertilization (Gallegly, 1968). Although some isolates form unequal numbers of oogonia and antheridia due to varying nutrient status of the isolates, there is no association between mating type and maleness or femaleness (Galindo and Gallegly, 1960).

In a few rare cases, *in vitro* production of oospores has been induced without direct contact of the compatible mating types by interspecific mating, or by chemical and physical stimulation. Interspecific mating has been observed *in vitro* when A2 mating types of *P. parasitica*, *P. palmivora*, or *P. capsici* were paired with A1 isolates of *P. infestans* (Shen et al, 1983). It is assumed that

chemicals or a single chemical can stimulate oospore formation without physical contact of hyphae. A1 and A2 isolates separated by a porous membrane to prevent mycelial contact formed oospores (Shattock et al, 1986a). An A1 isolate exposed to the substances emitted by an A2 strain formed oospores which germinated into A1, A2 and self-fertile colonies (Ko, 1994). Oospores have been produced *in vitro* by self fertilization of A1 mating types by placing cultures started from single hyphal tips near a polycarbonate membrane. The film apparently provided an environmental stimulus that provoked oospore formation. All five resulting progeny tested were A1 (Campbell and Duncan, 1985).

Oospores occasionally appear in single cultures of some isolates. Single lesions on plant tissue have rarely contained mixtures of two isolates (Matuszak et al 1994, Deahl et al 1993) and self-fertile cultures have been rarely found (Campbell and Duncan 1985, Fyfe and Shaw, 1992). A small number of field isolates which formed abundant oospores in the absence of a compatible mating type have been recorded in California, Japan, France and Mexico (Mosa et al, 1989, Shaw, 1987). These self-fertile cultures have been found to be either simple mixtures of A1 and A2 hyphae, or a unique genotype capable of forming viable oospores while promoting sexual and repressing asexual reproduction (Campbell and Duncan, 1985). Self-fertile isolates discovered at a low frequency from England and Wales were examined by Fyfe and Shaw (1992). They attempted to determine whether a self-fertile colony was a simple mixture of A1 and A2 hyphae or a single genotype by producing cultures from hyphal tip or single sporangia, and observing the behavior of the isolates when paired with A1 and A2 isolates. The progeny were primarily either A1 or A2 colonies; however, a few progeny grew into self-fertile colonies, thus behaving as a self-fertile phenotype (Fyfe and Shaw 1992). Tooley et al (1993) observed that two out of 35 isolates of *P. infestans* collected in Ireland from 1988-1989 were self-fertile,

forming abundant oospores in single culture. Vartanian et al (1985) observed that two out of 57 isolates of *P. infestans* collected from potatoes and tomatoes in southern California were self-fertile. A colony started from a sporangium produced oospores both in axenic culture and when paired with A1 and A2 mating types. At that time, the A2 mating type was unknown in southern California. The self-fertile isolates in both of these studies showed adpressed growth. However, it is difficult to use colony morphology alone to identify isolates because *P. infestans* has highly variable morphology (Caten and Day,1977).

The genetic determination of mating type has unfolded slowly due to the difficulty of germinating oospores in the lab, until Shattock et al (1986b) succeeded in developing a reliable protocol to induce germination. Progeny of *P. infestans* crosses appear to segregate to give varying proportions of A1 and A2 mating types which deviate from a 1:1 ratio. Shaw (1987) suggested that mating type is governed by two alleles of a single gene and since *P. infestans* is diploid, one mating type would be heterogametic (A2) and the other homogametic (A1). A1 could thus be derived from A2 by recombination during selfing or after mitotic crossing over. A2 could not be derived from A1 by this means (Shaw, 1987).

Oospore germination and infectivity

Oospore germination starts with thinning of the inner wall of the double walled oospore. The outer wall stretches as the oospore swells, and a germ tube originates from just under the outer wall. A single germ tube forms per oospore terminating with a single sporangium. The sporangium liberates zoospores which germinate and form colonies in the host (Gallegly, 1968).

The development of a reliable protocol for inducing germination of oospores was an important step in the investigation of *P. infestans* genetics and exploring the impact of sexual reproduction on the disease cycle. Oospores of *P. infestans* remain dormant for indefinite periods. Oospore germination has been induced by exposure to visible light (Gallegly, 1968) or a chilling period (Ribeiro, 1983). Oospores may also germinate as soon as they are formed in nature (Drenth et al, 1994c). Shattock et al (1986b) developed a reliable protocol for forcing oospore germination. Oospores were fed to snails, collected, then surface sterilized and placed on water agar. The oospores germinated three to five days later after continuous exposure to blue light with a white light background at 18° C. Pittis and Shattock (1994) conducted a series of experiments to demonstrate the variability in oospore germination. Oospores were observed on leaves four - nine days after inoculation with sporangia from A1 and A2 isolates. These oospores germinated after 14 days of incubation at 18°C. Oospores germinated successfully after immersion in sterile distilled water at 20°C. Longer dormancy periods increased the oospore germination potential (Pittis and Shattock, 1994).

Oospores remain viable over a wide range of weather conditions, but parentage affects viability of progeny. Oospores remained viable for at least eight months when exposed to natural weather conditions in the Netherlands (Drenth et al, 1994c). High temperatures limited oospore survival to less than two days at 40°C. There was good oospore survival at temperatures ranging from -80°C to 35°C (Drenth et al, 1994c). Oospore viability was highly variable, however, between progeny of different crosses. Pittis and Shattock (1994) demonstrated that oospores could produce lesions on susceptible potato shoots. Zoospores from germinating oospores may tend to infect the stem and lower canopy. This can present disease management problems, since the lower haulm

is typically a difficult area to cover during pesticide application. Oospore build up in the soil could lead to crops showing widespread infection very early in the growing season or even prior to plant emergence (Shaw, 1987).

Distribution of mating types

The A1 mating type has been ubiquitous where potatoes are grown since the first proposed *P. infestans* migration from Mexico to Europe in the 1800's. The first report of the A2 mating type outside of Mexico did not occur until 1984 (Hohl and Iselin, 1984). The A2 mating type has now been reported from B. C. (Deahl et al, 1992), the United States (Goodwin and Fry, 1994), Great Britain (Malcolmson, 1985), the Netherlands (Fry et al, 1991), Norway (Hermansen and Amundsen, 1995), Switzerland (Hohl and Iselin, 1984), Ireland (Tooley et al, 1993), Germany, Poland (Daggett et al, 1993), Japan, South Korea (Mosa et al, 1989), Israel (Grinberger, et al 1989), Arab Republic of Egypt (Shaw et al, 1985), and in the northern hills region of India (Singh et al, 1994). Although France is encircled by countries where the A2 mating type has been observed, it has not been observed among isolates collected from several French regions from 1988 to 1992 (Andrivon et al, 1994).

The distribution of the A2 mating type and its frequency relative to the A1 mating type has increased rapidly over a short period of time. It is assumed that the A2 mating type was only introduced to Europe on increased numbers of shipments of potatoes from Mexico during 1976 (Fry et al, 1993). A higher occurrence of A1 over the A2 mating type would be expected as it is the original established population. However, the opposite has been observed in Poland, Egypt, Israel, India and Korea. The A2 mating type was not observed in Eastern Germany and neighbouring Poland until 1980. The A2 mating type now appears to be established in Poland, appearing in all fields sampled (Daggett et al, 1993).

The initial discovery of the A2 mating type in Egypt revealed that all 88 of the *P. infestans* isolates collected from exported table potatoes were of the A2 mating type (Shaw et al, 1985). A lower than expected frequency of A1 was found in fields in Israel from 1983 to 1988 (Grinberger et al, 1989). The A2 mating type was predominant in samples taken from Ontario, Quebec and New Brunswick (N. B.) in 1994 (Platt et al, 1995). The trend of higher frequency of A2 was also seen in Asia and the Far East. In India, the frequency of A2 isolates discovered in the Shillong and Darjelling Hills increased during 1991 to 1992 (Singh et al, 1994). Isolates of *P. infestans* analyzed for mating type in Japan and South Korea were predominantly A2 (Koh et al, 1994). Half of the 30 Japanese isolates were the A2 mating type and 56 out of 57 South Korean isolates were A2. This rapid increase in the frequency of the A2 mating type may be attributed to a higher fitness level resulting in displacement of the old population characterized by the A1 mating type (Fry et al, 1993).

Molecular characterization

The development of molecular techniques for genotypic characterization of *P. infestans* can provide valuable insight into the characteristics of changing populations. Randomly amplified polymorphic DNA markers (RAPD) show excellent potential for genetic analysis of *P. infestans*, but a marker has not yet been developed (Maufrand et al, 1995). Allozyme gels, nuclear DNA fingerprinting, and restriction fragment length polymorphism (RFLP) of mitochondrial DNA are currently being used to identify genotypes of *P. infestans* isolates collected since the 1970's (Fry et al, 1993). This characterization is based on identification of the loci of glucose-6-phosphate isomerase (Gpi), malic enzyme and peptidase (Pep) loci in combination with the nuclear DNA fingerprint

(Fry et al, 1993, Tooley et al, 1985). The isozyme analysis is useful because the alleles coding for the enzymes segregate independently and are expressed regardless of the environment in *P. infestans* (Tooley et al, 1985, Chang and Ko, 1991). These genotypic characteristics are used in combination with mating type and metalaxyl sensitivity to compare genetic diversity in populations (Goodwin et al, 1994b, Spielman et al, 1991).

Genetic markers indicate a high diversity in the population of *P. infestans* in Mexico. In central Mexico, almost every isolate has a unique genotype (Fry et al, 1993, Goodwin et al, 1992). The comparison of genotypic characteristics of *P. infestans* isolates collected from Asia, Europe, North Africa, and North America prior to 1980 shows a close similarity. This genotype is characterized by the A1 mating type, metalaxyl sensitivity, and the same Gpi and Pep loci, and DNA fingerprint, and thus is assumed to be from a single clonal lineage.(Fry et al, 1993). This group has been named the US-1 clonal lineage (Goodwin et al, 1994a). The changes in severity of late blight in Europe, North America and North Africa since 1980 are attributed to the establishment of "new" clones of *P. infestans* (Fry et al, 1993). These new populations were probably introduced to Europe on potato tubers shipped from Mexico in 1976 (Fry et al, 1993, Goodwin et al, 1992). In South East Asia, two genotypes have been identified in the population of *P. infestans* in Japan and South Korea. One genotype is identical to the "old" clonal population and the other is unique leading to the assumption that it has probably originated from separate immigration from Mexico rather than via seed piece transfer from Europe or North Africa (Koh et al, 1994).

The population in the United States and Canada has also become more diverse. Prior to the mid 1980's, it was also represented by the clonal lineage US-1, characterized by the A1 mating type and limited genetic variation (Goodwin et al, 1994a). From 1987 to 1991, a total of 18 genotypes (including

variants of genotypes by one locus) based on allozyme and DNA fingerprint and mating type have been identified from isolates collected in Canada and the United States. These include the "old" clonal lineage US-1 and new populations named by clonal lineages US-5 and 6 (both A1 mating type), US-7 and 8 (both A2 mating type), BC-2 and BC-3 (both A2 mating type) and CDA 1 (A1 mating type) (Goodwin et al, 1994a) The US-6 genotype was the most common genotype found on isolates from 1987 to 1991 (Goodwin et al, 1994a). BC-2 and BC-3 are found only in B. C., and CDA 1 was found in N. B. in 1980. This new population includes both the A1 and A2 mating types and metalaxyl resistant and sensitive isolates. US-5 has been found instead of US-6 in the state of Maine (Goodwin et al, 1994a). In 1994, US-8 (A2 and metalaxyl resistant) was found in several eastern states, as well as in N. B. (Goodwin and Fry, 1994, Goodwin et al, 1995). There has been no evidence of genetic variation due to sexual recombination among isolates collected from the United States and Canada between 1979-1991 thus it is assumed that the "new" genotypes are clones of introductions from Mexico. (Goodwin et al, 1994a).

Methods for disease control

Late blight is controlled on potatoes by a number of methods: sanitation, quarantine and fungicide application. Sanitation involves the destruction of cull piles and roguing of overwintering field culls which could be spring volunteers to reduce the load of overwintering pests. Top-killing of vines two weeks prior to harvest also kills *P. infestans* colonies due to lack of adequate substrate, thus preventing inoculation of tubers from infected vines during harvest (B.C.M.A.F.F., 1995). Potato crops are vegetatively propagated and the tubers provide an excellent substrate for obligate pathogens like *P. infestans*.

Sanitation in storage requires rapid identification of the pathogen because late blight infected tubers rot in storage, providing an avenue for bacterial infection resulting in rapid deterioration of tubers and great storage loss. A PCR (polymer chain reaction) primer has been developed for rapid detection of *P. infestans* (Tooley and Carras, 1995). This primer has potential for use in detecting seedlot infection, which can reduce disease spread by infected seed. Spread of *P. infestans* can also occur during washing of tubers before sale as prepackaged potatoes. The sporangia in the wash water are not completely killed by addition of disinfectant to water, but heat treatment effectively prevented infection of healthy tubers (Fairclough et al, 1995).

Potato late blight is not a quarantinable disease; however, in 1994, a two year pilot program to eradicate late blight from the Pemberton Valley was implemented. The program involved restriction of movement of tomato plants into the valley, as well as inspection of fields with zero tolerance for late blight (D. Ormrod, personal communication). Since the fungus has a wide host range of solanaceous plants, including hairy nightshade (*Solanum sarrachoides* L.), tomato (*Lycopersicon esculentum* Mill.), and pepper (*Capsicum annum*), disease control on other hosts is important in disease eradication (Vartanian and Endo, 1985).

Fungicide use

Fungicide application to prevent late blight is necessary to successfully produce a potato crop because of the potential for rapid asexual reproduction of the pathogen throughout the host's growing season (Parry, 1990). Sprayers must be calibrated and operating efficiently to deliver thorough fungicide coverage. High pressure must be used to ensure complete coverage of the lower canopy during mid to late season when the canopy is dense. The

development of a "dropleg" sprayer which enables the crop to be sprayed from the bottom as well as from the top with a conventional boom sprayer has reduced stem and tuber infections due to late blight (Ligertwood and Hinds, 1995). This may become increasingly important if zoospores released from germinating oospores can infect the lower haulm.

All fungicides are formulated as plant protectants, and not to eradicate established infections. The non-systemic fungicides serve primarily as a toxic barrier with very limited or no penetration into the plant and are thus immobile and subject to the effects of weathering (Sisler, 1977). Protectant, non-systemic fungicides act rapidly on a number of sites within the pathogen, thus limiting the ability of fungus to detoxify the fungicide. Maneb, for example, interferes with the sulfhydryl protein groups, its other fungi-toxic mechanisms are not understood (Fry,1982). The fungicides which are applied as protectants against *P. infestans* in B. C. are Bravo 500 (chlorothalonil), fixed copper (copper oxychloride), Dithane M-45 or Dithane DG or Manzate 200 DF (mancozeb), Maneb or Dithane M-22 (maneb), Polyram DF (metiram), Zineb (zineb) and Ridomil/MZ 72 W (mancozeb 64% / metalaxyl 8 %) (B.C.M.A.F.F., 1993). Ridomil/MZ is the only systemic fungicide registered for protection of potatoes from late blight. To minimize resistance build-up, the systemic fungicide dose should be as high as possible and combined with a second fungicide to prevent growth of resistant mutants in the population. The choice of the second fungicide should be made with consideration for minimizing cross resistance development. A non systemic fungicide appears to be more effective in combination with a systemic fungicide. Metalaxyl has no effect on zoospore or sporangia germination compared to a protectant fungicide like maneb, which prevents sporangia and zoosporangia germination (Staub et al, 1980). Mancozeb is added to the Ridomil formulation since metalaxyl does not inhibit spore germination. Fungicides such as

mancozeb and folpet inhibit energy production and are strong inhibitors of zoosporogenesis in *P. infestans* (Bashan et al, 1989). The addition of a third fungicide, cymoxanil, to the metalaxyl - mancozeb mixture, has prevented build-up of metalaxyl resistance when used in combination with strict timing and control of the number of metalaxyl applications (Collier and LeBoutillier, 1995). To minimize risk of resistance build up, Ridomil MZ is recommended for use only three times during the season, and only when late blight is not obviously present in the field (B.C.M.A.F.F., 1993).

Preventive measures should be used to protect potato fields near mulch covered early spring potato crops from late blight. The floating mulch placed over the crop to hasten maturity also provides an ideal environment for *P. infestans* growth. Crops covered with floating mulch should be isolated from neighboring blight susceptible crops. If floating mulch is used, neighbouring potato or tomato fields should be sprayed with a protectant fungicide to prevent pathogen spread to neighbouring fields from inoculum remaining on the vines of the harvested crop.

The two approaches to fungicide application against late blight are based on crop growth (calendar sprays) or disease forecasting. The calendar spray program begins with an initial fungicide application when the plant canopy meets over the rows. In the lower Fraser Valley, however, the first recommended spray is copper oxychloride after hilling (D. Ormrod, personal communication). This spray provides a fungi-toxic layer of copper on the soil which limits the survival of sporangia and zoosporangia which fall to the soil throughout the growing season. Fungicides are then applied at 10 - 14 day intervals to protect the new foliage, and to replace protectant fungicide lost due to weathering. Systemic fungicides have been particularly useful since they are not subject to weathering and give more protection to new growth.

Disease forecasting

The destructive impact of late blight has led to the development of several sophisticated disease forecasting systems based upon the environmental conditions that limit pathogen growth, stage of crop growth, local fungicide resistance and host plant disease resistance (Shtienberg et al, 1995). Monitoring systems incorporate meteorological data such as temperature, duration of rain fall, leaf wetness and relative humidity. Since zoospores require water to move about, relative humidity is sometimes used to infer leaf wetness duration (Van der Plank 1975). Late blight can become epidemic when the temperature is no lower than 10°C and the relative humidity is above 90% for at least 11 hours per day (Parry, 1990). These conditions encourage zoospore development, thus instead of a single sporangium starting a lesion, zoospore release from a sporangium could produce six to eight lesions, resulting in a rapid increase in diseased plant area. The general weather data can provide an indication of outbreak conditions and has provided the basis for development of disease forecasting systems.

The micro-climate of the crop canopy determines the potential severity of disease. Early in the season before the rows are filled in and the foliage is sparse, an epidemic is less likely to occur as solar radiation and wind dry foliage rapidly (Van der Plank, 1975). Later in the season, dense foliage coupled with early morning fog or dew can increase the potential of an epidemic occurring since the lower haulm is less likely to dry out.

A late blight forecasting system has been in use in the lower Fraser Valley since 1980. The system was implemented initially because farmers felt that fungicide applications on a calendar basis were excessive, and not because of potato late blight epidemic control problems (B. Vernon, personal

communication). The forecasting system can result in decreased fungicide applications as the growers only apply fungicides when the weather is conducive to disease development. Many growers employ crop monitoring companies to monitor their crops for insect pests and diseases such as late blight. Late blight is monitored by placing a weather station on a farm to monitor local temperature and relative humidity to make estimates of the late blight hazard. The late blight hazard index used was developed by Agriculture Canada specifically for the Fraser Valley. The model, based on relative humidity, temperature and rainfall, is applied to several variables including cultivar, stage of growth, presence or absence of blight, potential of oospore presence, metalaxyl resistance and the two day weather forecast (B. Vernon, personal communication). The information is then used to determine a spray schedule for the individual farm.

The existence of both mating types portends sexual reproduction, with the possibility of increased variation in the pathogen resulting in changes in virulence and fungicide tolerance. Late blight management strategies have been based on knowledge of the environmental conditions required for development of sporangia and zoosporangia (Stevenson, 1993). Sanitation practices and pesticide applications have been successfully used in combination with blight forecasting systems to prevent epidemics. However, oospore production could result in reduced efficacy of the blight management system due to the unpredictable germination of oospores (Ribeiro, 1983). Since oospore viability is decreased at 40°C, soil temperature monitoring as well as soil solarization as part of a crop rotation schedule could provide inoculum control and monitoring in fields where oospore contamination is suspected (Drenth et al, 1994a).

Fungicide resistance

Generally, fungicide resistance is uncommon with broad spectrum fungicides. Fungicide resistance became a concern with the introduction of narrow spectrum, single- site mode of action systemic fungicides (Dekker, 1976). Simple mutations in the pathogen at sites targeted by the latter type of fungicide can lead to the development of fungicide resistance.

Mechanisms of fungicide resistance may be based on detoxification or avoidance of the toxin. One mechanism of fungicide resistance can be due to a decrease in membrane permeability, which prevents the toxicant from reaching target sites. Antimycin A toxicity is averted because the wild type of the fungus has two different electron transport routes in the respiratory chain, while the sensitive mutant with only one electron transport route (Dekker, 1976). Fungi also may detoxify the fungicide by converting the toxin to a less fungi-toxic form. The toxic effect can also be circumvented completely, as in the example of antimycin resistance in *Ustilago maydis* (Dekker, 1976).

The paradigm of fungicide resistance is that resistant mutants generally decrease rapidly in the population unless the selection pressure persists. This has been observed in experimentally produced mutants; however, naturally occurring resistant individuals often persist (Fry, 1982). Fungicide resistant mutants show a wide range of variation in competitive qualities. There is evidence that some resistant mutants exhibit increased survival abilities even in the absence of the fungicide. Benomyl resistant strains of *Verticillium malthousee*, for example, were isolated in the Netherlands before the introduction of benomyl (Dekker, 1976). Fungi which are able to spread rapidly, such as those which propagate via air-borne spores, generally become a problem more rapidly when resistant isolates are found than do the slower spreading fungi.

Metalaxyl resistance

Metalaxyl belongs to the phenylamide group of fungicides used to control plant diseases caused by *Peronosporales*. Metalaxyl specifically inhibits rRNA synthesis in sensitive strains of *P. infestans* but the specific target site has not yet been identified (Davidse et al, 1991). Metalaxyl does not prevent sporangial germination. Since it is systemic, it affects the fungus after initial haustorial penetration by inhibiting further development. Metalaxyl is taken up by roots if applied as a soil drench and by foliage if applied as a foliar spray. It is transported acropetally within 24 hours of application (Cohen and Coffey, 1986).

Resistance to metalaxyl is acquired through mutation in some *Peronosporales*. *P. capsici* and several species of *Pythium* lost their resistance to metalaxyl during repeated transfers on agar (Bruin and Edgington, 1981), indicating that metalaxyl resistance is acquired through mutations. *P. infestans* isolates, however were stable in metalaxyl resistance over several transfers (Davidse, 1982). Multinucleate fungi such as *P. infestans* show a wide variation in fungicide tolerance. Metalaxyl sensitivity in *P. infestans* varies among isolates, leading to the use of the term insensitive with regard to metalaxyl (Matuszak et al, 1994, Koh et al, 1994). Varying degrees of sensitivity usually exist for different loci. In heterokaryotic cells, the resistance characteristics are not always expressed immediately, and expression is varied, either dominant, recessive or semidominant. Metalaxyl was tested extensively to determine the rate at which selection for resistance of *P. infestans* would occur by subjecting isolates to sub-lethal doses of metalaxyl. Isolates retained sensitivity for over 12 continuous generations when subjected to selection pressures for resistance (Davidse, 1982). Metalaxyl insensitive isolates of *P. infestans* have been found

in collections predating the introduction of the fungicide, indicating that metalaxyl insensitivity may occur naturally at a low level in the wild population. Metalaxyl insensitive Mexican and German isolates collected before metalaxyl was used have been found (Matuszak et al, 1994, Daggett et al, 1993). However; given the great mobility of *P. infestans* due to aerial transmission, it is more likely that insensitive isolates were introduced to areas where metalaxyl was not yet used. Metalaxyl insensitive isolates of *P. infestans* were also insensitive to the systemic fungicides cyprofuram, folpet, promocarb and phosethyl AL, which are all active against Oomycetes (Cohen and Samoucha, 1984). The basis for metalaxyl insensitivity in *P. infestans* is not fully understood, but it appears to involve several genes, or has a cytoplasmically inherited component (Shaw and Shattock 1991). This could explain the durability of metalaxyl insensitivity in the population of *P. infestans*.

Screening for fungicide insensitivity

The first step in quantifying fungicide insensitivity is to determine a baseline sensitivity using data from the literature and establishing the reaction of the wild types (Georgopoulus, 1982). The method used to measure insensitivity should then give a reliable description of insensitivity. Various tests have been used to provide qualitative and quantitative estimates of fungal reaction to fungicides. The effect of fungicides on spore germ tube elongation in media containing fungicides has been used to give a qualitative estimate of insensitivity. The rate of increase in colony diameter in response to different concentrations of fungicide can provide a quantitative measure of insensitivity. Obligate parasites, such as powdery mildew, have to be tested for fungicide insensitivity on living plants. These tests use entire plants or leaves sprayed with fungicide, or leaf discs placed on fungicide to provide a qualitative estimate

of insensitivity (Sozzi and Staub, 1987). Although *P. infestans* is an obligate parasite in nature, it can be grown on rye extract or V-8 media (Caten and Jinks, 1968).

Sensitivity of *P. infestans* to metalaxyl can be tested by a number of quantitative and qualitative methods, including fungal growth response on plants sprayed with fungicide, placing tuber or leaf discs on solutions of varying concentrations of metalaxyl, or colony growth on agar supplemented with metalaxyl. Several concentrations of metalaxyl are used to provide a dose response curve. The tuber or leaf discs of potato are either floated on a metalaxyl solution or placed on a filter paper soaked in metalaxyl solution, a droplet of inoculum is then placed on the surface of the potato and incubated for up to seven days (Dowley and O'Sullivan, 1985, Kadish and Cohen, 1988). Sensitivity is determined by presence or absence of a sporulating colony on the tuber discs or agar, or absence or presence of complete necrosis of the leaf disc. The low water solubility of most fungicides is the most common source of error in testing of isolates (Georgopoulus, 1982). Sensitivity is also tested by placing an agar plug of a colony on agar amended with metalaxyl and measuring the colony diameter after seven to 10 days and comparing it to the diameter of the isolate grown on a control medium (Davidse et al 1981). Metalaxyl sensitivity is then expressed as percent growth on metalaxyl over growth in the control. This method provides a quantitative estimate of the range of metalaxyl sensitivity. These methods provide similar qualitative estimates of fungicide sensitivity; however, the slope of the dose response curve from the agar plate assays shows less differentiation between sensitive and insensitive isolates than the slope of the dose response curve formed by the tuber or leaf disc assays (Sozzi and Staub, 1987).

Managing fungicide insensitivity

The development of metalaxyl insensitive *P. infestans* populations is well documented in the Netherlands (Davidse, 1982). Metalaxyl was first introduced to the Netherlands in 1979. In 1980, the weather conditions were very favorable for development of a late blight epidemic. About 50% of the fields received metalaxyl through application of Ridomil 25WP. Dutch farmers reported poor performance of metalaxyl for late blight control in fields sprayed with metalaxyl alone. Later, metalaxyl was combined with a non systemic fungicide, mancozeb, but disease control was still inadequate (Davidse, 1982). The majority of isolates collected were found to be metalaxyl insensitive. Intense sampling from the same fields yielded isolates which were either all insensitive or all sensitive, except in one case where the isolates were taken from a field next to an unsprayed plot, where the sensitive isolate was assumed to have originated (Davidse et al 1981). Widespread insensitivity to metalaxyl appeared very rapidly, perhaps due to initial extreme selection pressure through metalaxyl use without a non-systemic fungicide.

Cohen and Samoucha (1989) showed that mixing metalaxyl with one or two companion fungicides reduced the rate of buildup of insensitivity and improved disease control, but did not prevent the insensitive sporangia from dominating the fungal population at the end of the epidemics. The rate of insensitivity build up was more dependent on the initial frequency of insensitive sporangia in the inoculum than on fungicide use. Crops that were not treated with metalaxyl had a relatively slower rate of increase in metalaxyl insensitivity build-up than crops that were treated with metalaxyl containing fungicides. An uncontrolled epidemic resulted in a final population of 89-92% insensitive sporangia (Cohen and Samoucha, 1989). Insensitive isolates appeared to have an advantage over susceptible isolates, even in the absence of selection

pressure for insensitive isolates. Fungicide application delayed, but did not prevent, insensitivity development in the populations.

Relationship of mating type and metalaxyl sensitivity

Since the introduction of metalaxyl insensitivity has almost coincided with the movement of the A2 mating type out of Mexico, the relationship of the two phenotypes has been of interest. Metalaxyl response of isolates collected in eastern Germany between 1976 - 1990 showed an increase in the level of metalaxyl insensitivity. The majority of insensitive isolates were of the A1 mating type and a significant correlation was found between the A1 mating type and metalaxyl insensitivity (Daggett et al, 1993). Analysis of Polish isolates collected in 1989 also showed a significant correlation between the A1 mating type and metalaxyl insensitivity (Therrien et al, 1993). A correlation between the A2 mating type and metalaxyl insensitivity has been observed in isolates (clonal lineages US-7 and US-8) obtained from the United States and Canada (Goodwin and Fry, 1994). There was a great deal of variation in the metalaxyl sensitivity of Japanese A2 isolates (Koh et al, 1994), although nuclear DNA fingerprinting indicated that all the Japanese A2 isolates were of the same genotype, JP-1 (Koh et al, 1994). Korean isolates were predominantly A2 and of low to intermediate sensitivity to metalaxyl (Koh et al, 1994). The great variation of metalaxyl sensitivity and mating type of *P. infestans* isolates in different geographical locations appears to indicate that it is unlikely that mating type and metalaxyl sensitivity are linked.

Fitness

"Fitness" describes the ability of a species to reproduce successfully and survive indefinitely within its habitat. Success is determined by the organism's ability to produce offspring which in turn can reproduce. Fit organisms are able to develop in and effectively exploit their habitat for nutrition, and survive adverse weather conditions. The fitter organisms are able to dominate within their environment, ultimately displacing the less fit individuals. The rapid displacement of the "old" population as well as the increased severity of late blight outbreaks imply that the new population has fitness advantages over the older population (Fry et al 1993), although there is as yet no explanation for this.

P. infestans has been demonstrated to be a highly variable species in terms of growth rate (Caten and Jinks, 1968). The various fitness qualities of the new population of metalaxyl insensitive *P. infestans* are being studied in attempts to understand its success. Caten and Jinks (1968) observed the characteristics of colonies started from zoospores compared to colonies started from sporangia or from single hyphal tips of a clonal population of *P. infestans*. Subcultures started from zoospores were more variable than cultures started from hyphal tips or sporangia.

Fitness of metalaxyl insensitive strains

Fungal insensitivity to some fungicides has been linked to reduced fitness. The development of insensitivity has been associated with a loss in virulence of a fungus due to slower growth or an inability to overcome host resistance. Laboratory selection for pimaricin resistant yeasts and fungi has resulted in fungi with reduced sporulation and pathogenicity (Dekker 1982). This is not the case with all fungi. Metalaxyl insensitive strains of *Phytophthora nicotianae*, *P.*

megasperma f.sp. glycinea as well as *P. infestans* have not shown reduced fitness (Dekker 1982).

In the absence of selection pressure, isolates of *P. infestans* from a metalaxyl insensitive population in Israel were more infective to potato foliage than isolates from a metalaxyl sensitive population during relatively short periods of leaf wetness (Bashan et al, 1989). Metalaxyl insensitive isolates produced zoospores more rapidly than sensitive isolates. Metalaxyl insensitive isolates had enhanced oxygen consumption which was related to higher rates of metabolism and faster zoospore production but no significant difference was detected in the mycelial respiration or *in vitro* linear growth between the sensitive and insensitive isolates (Bashan et al, 1989). Holmes and Channon (1984), however, were unable to detect differences in spore production between metalaxyl sensitive and insensitive isolates. The number of spores produced with all the treatments varied considerably, apparently due to physiological differences in the leaves used to grow the colonies.

The fitness attributes which make metalaxyl insensitive *P. infestans* successful vary according to the region from which the tested isolates originated. Laboratory and field observations have resulted in contradictory evidence regarding the relative fitness of insensitive and sensitive strains of *P. infestans*. Insensitive isolates in Ireland were less fit than the sensitive isolates. Metalaxyl was introduced for commercial use in Ireland in 1977, and by 1980, insensitivity was widespread and resulted in withdrawal of metalaxyl from the market (Dowley, 1982). Within three years, the frequency at which insensitive isolates were detected in the field decreased from 75% to 6%. The metalaxyl sensitive strain produced more sporangia per unit leaf area than the insensitive strain (Dowley, 1987). It appears that the insensitive population in Ireland was different in its fitness level than the insensitive population in Israel. Immigration

and overwinter survival of the different individuals is an important consideration in fitness evaluation.

Dekker (1982) proposed that differences in fitness of sensitive and insensitive isolates may be determined by inoculating a plant with a mixture of insensitive and sensitive spores. The ratio of sensitive to insensitive conidia collected after lesion formation would determine which strain was more competitive under experimental conditions. However, field observations are necessary to get a complete answer. The contrasting results with *P. infestans* in terms of linking fitness to fungicide insensitivity makes it difficult to generalize. The variable reactions and morphology accentuate the importance of field testing populations from different regions.

The ability of strains to survive harsh weather conditions adds another parameter to fitness evaluation. It has been assumed that *P. infestans* mycelium survives the winter in potato tubers and other plant debris and resumes growth when weather conditions permit. The appearance of the A2 mating type in North America, North Africa, Europe and Asia may introduce oospores as an overwintering phase. The starchy potato tuber and plant debris from other hosts such as *Solanum sarrachoides* Sendtner ex. Mart (hairy nightshade) can provide overwintering habitats for mycelium of *P. infestans* (Vartanian and Endo, 1985). The overwintering of metalaxyl sensitive and insensitive isolates was studied in Israel (Kadish and Cohen, 1992). The metalaxyl sensitive isolates tended to overwinter better than the metalaxyl insensitive isolates, because the metalaxyl insensitive isolates were so aggressive that they either mummified or rotted the entire potato, thus preventing sporulation when weather conditions were favorable. Thus, rapid growth, which is a fitness advantage with regard to pathogenesis of host tissue, was a disadvantage in terms of winter survival.

However, the rapid worldwide increase in the occurrence of metalaxyl insensitive isolates suggests that it has some fitness advantages over the sensitive isolates.

The need for research

In recent years, increased incidence and severity of potato late blight has been reported in many potato growing regions around the world (Deahl et al, 1993, Fry et al, 1993, and Goodwin et al, 1994a). The increased prevalence of late blight may be attributed to a combination of several factors: (i) the occurrence of recently introduced or immigrant genotypes that have displaced original populations (Fry et al, 1993, Goodwin et al, 1994a, Spielman et al, 1991); (ii) the occurrence of the A2 mating type, first detected in the United States in 1987 and in Canada in 1991 (Deahl et al, 1991, Fry et al, 1993); and (iii) the occurrence of insensitivity of the pathogen to the systemic phenylamide fungicide metalaxyl (Deahl et al, 1993, Fry et al, 1993).

In Canada, the prevalence of late blight has also increased in recent years. Ridomil 25W was used in B.C. from 1980 - 1982, by 1983 it had been substituted with Ridomil MZ72. Late blight lesions were tested every autumn for metalaxyl sensitivity from 1980 - 1985. All isolates tested were metalaxyl sensitive (D. Ormrod, personal communication). During the summer of 1989, the late blight forecasting system used in the lower Fraser Valley was ineffective in controlling a late blight epidemic (B. Vernon, personal communication). An isolate of *P. infestans* collected from Richmond, B. C., was identified as the A2 mating type and insensitive to metalaxyl, representing the first report of the A2 mating type in Canada (Deahl et al, 1991). An additional four isolates collected from potato fields in B. C. during 1991 - 1992 were found to represent unique genotypes of the pathogen (Goodwin et al, 1994a). Insensitivity to metalaxyl was also suspected, and the discovery of the A2 mating type with a unique genotype (from an isolate collected in the lower mainland) confirmed that the population had indeed changed (W. E. Fry, written communication). There was a lack of information on the prevalence of the A2 mating type and metalaxyl

sensitive isolates in B. C. and the rest of Canada. It was important to determine the prevalence of these characteristics within Canada and to assess the possibility of a changing population of the pathogen as had already been noted in other parts of the world (Fry et al, 1993, and Spielman et al, 1991).

Objectives of research

The objectives of the research described in this paper were to:

(1) characterize isolates of *P. infestans* from samples collected from several provinces in Canada for sensitivity to metalaxyl *in vitro* ;

(2) determine the mating type of isolates of the pathogen;

(3) monitor changes in the ratios of sensitive: insensitive isolates and of A1:A2 mating types during the growing season;

(4) determine the influence of metalaxyl application, potato cultivar, and time of sampling on population characteristics of the pathogen; and

(5) compare isolates' capacities for growth on agar, and colonization, sporulation and survival on potato tissues.

CHAPTER 2

MATERIALS AND METHODS

Source of isolates

During the 1993-1994 growing seasons, late blight-infected potato leaf samples were obtained from Alberta (provided by R. Howard, Alberta Agriculture, Brooks, and V. Bisht, Alberta Tree Nursery and Horticulture Centre, Edmonton), from British Columbia (B. C.) (collected by the author and laboratory technicians and also provided by C. Duff, P. Froese, and B. Vernon, Agriculture Canada; D. Henderson, E.S. Cropconsult Ltd., Vancouver; D. Ormrod, B.C. Ministry of Agriculture, Cloverdale; B. Peterson, Pro-Tect Service, Langley; B. Warner, B.C. Ministry of Agriculture, Sidney), from Manitoba (provided by L. MacDonald and G. McKenzie, Ciba Canada, Warren), New Brunswick (N. B.) (provided by G. Bernard, Department of Agriculture, Florenceville), and Prince Edward Island (provided by M. Drake, Island Crop Care Services, Charlottetown). Samples were collected during the period June 2 to September 23, 1993. In addition, cultures of *P. infestans* originating from Nova Scotia, Ontario, Prince Edward Island and Quebec (provided by G. White, Agriculture Canada, Ottawa) were included in the study. A small number of samples from infected tomatoes in home gardens in B.C. was included.

During 1994, isolates originated from Alberta, B. C., Manitoba, N. B. and Prince Edward Island and were collected during the period May 30 to September 10. The geographic locations within each province from which samples were obtained are indicated in Table 2 (see Results section). The sampling areas in B. C. were located in four geographically separated regions (Figure 1). The Okanagan Valley, the Pemberton Valley, and the Fraser Valley,

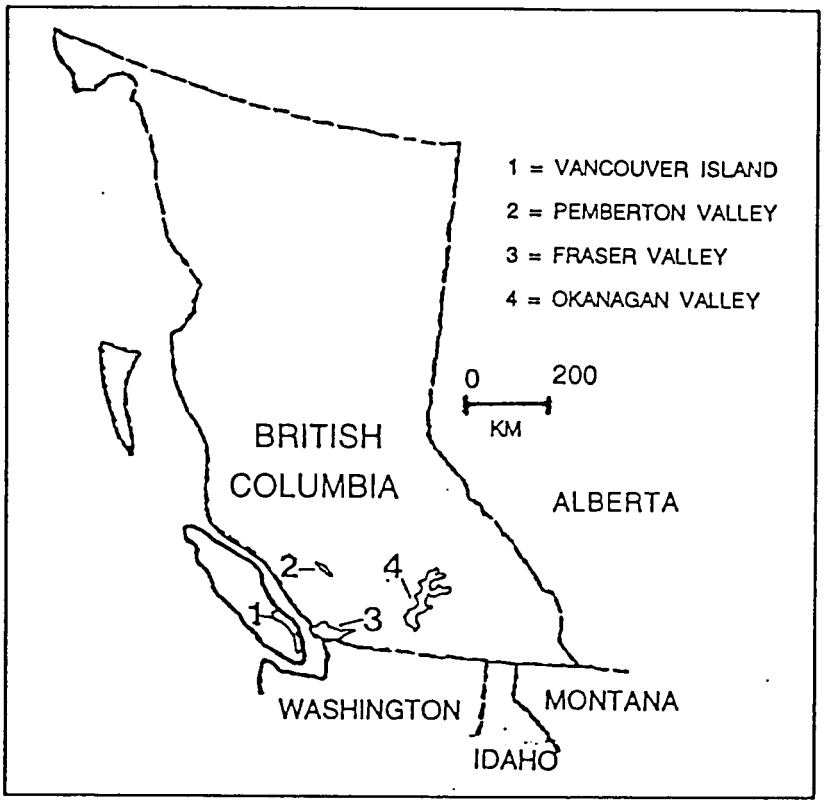


Figure 1. Geographic locations within British Columbia from which samples of diseased potato plants were obtained during 1993-1994.

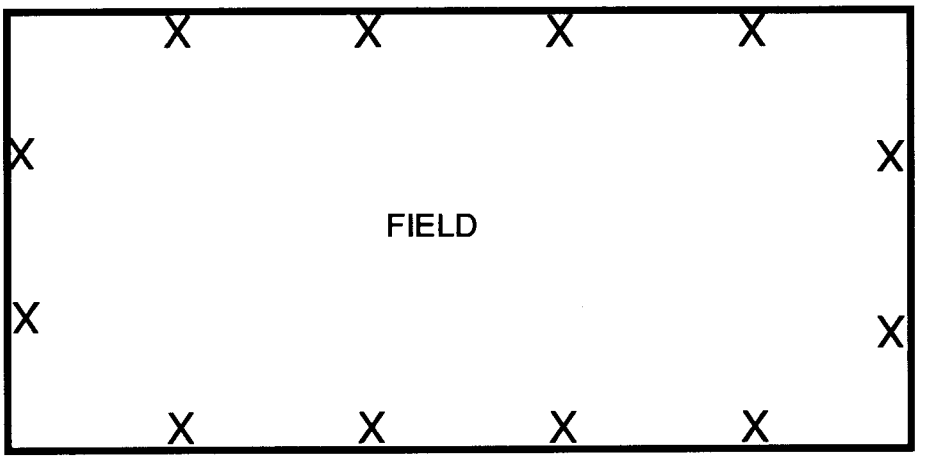
are each separated by a distance of about 140 km, while Vancouver Island is about 50 km to the west of the Fraser Valley (Figure 1). The Fraser Valley, where the most intensive sampling was done, included the rural areas of Chilliwack, Cloverdale, Delta, Ladner and Richmond. The agricultural area of Cloverdale is about 25 km from Delta, Ladner and Richmond. In Alberta in 1993, all samples were from the southern irrigation district near Brooks. In 1994, the samples were from the north-central region near Edmonton (Table 2, see Results section). In N. B., in 1994, samples originated from various parts of the province.

Sampling procedure and isolation methods

The potato fields sampled in B.C. ranged in size from 0.2 to 24 hectare, with an average of four hectare. When late blight lesions first appeared in a field, an average of 12 plants/field were sampled at evenly-spaced locations close to the field perimeter. If diseased plants were aggregated, effort was made to sample different plants at individual locations in the field. During subsequent resampling of the same field, an additional four diseased plants were also sampled at locations 10 meters from the perimeter (Figure 2). From each plant, a stem or leaflet from a compound leaf with lesions was collected and comprised one sample. A total of 12 or 16 plants/field were represented at each of the sampling dates, which varied from one to four, depending on the field. The total number of fields sampled and the months when samples were obtained are shown in Table 1. Fields were resampled at 15-55 day intervals depending on whether the disease was present. From each field, information was obtained on the cultivar grown and whether or not metalaxyl had been applied, and the overall incidence of disease in the field was estimated.

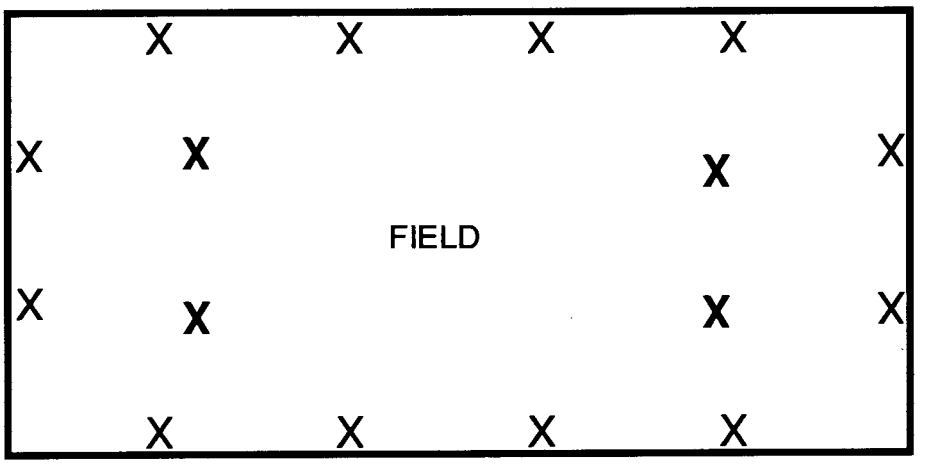
X - sampling location

1) First Sampling



Initial field sampling was carried out as above, taking 12 samples from evenly spaced locations around the field perimeter

2) Second Sampling



Subsequent sampling included taking four additional samples from within the field (10 meters in from the edge)

Figure 2. Field sampling strategies

Tissue pieces from lesioned areas were usually plated within 24-48 hours after collection; however, for leaf samples originating from out-of-province that were shipped, a delay of five to seven days could occur. All leaf samples were placed in plastic bags after collection and transported over ice in a cooler. Tissue pieces from the margin of lesions were plated onto rye agar (extract from 60 g rye grains boiled in one litre of distilled water, 20 g sucrose, 15 g agar) amended with 25 μg/ml nystatin, 100 μg/ml vancomycin and 20 μg/ml rifampicin (Caten and Jinks 1968, Shattock et al 1986). An average of six to eight tissue pieces/plant were plated on one petri dish (Figure 3). Occasionally, leaves were placed on freshly cut halves of potato tubers and incubated for five to seven days at 16 C before isolation was attempted. In 1994, isolations were also made onto 2% V8 agar amended with the same antibiotics as above. All petri dishes were incubated in the dark at 16-18 C for seven -14 days and then examined for colonies of *P. infestans*. Subcultures (mass transfers of mycelium) were made onto fresh rye agar or V8 agar containing antibiotics and incubated for two to eight weeks, at which time a third subculture was made. Penicillin G (200 μg/ml) was added to minimize growth of contaminant bacteria in some cultures. From each field sampled, an average of four to eight isolates was maintained, with each isolate originating from a different plant within the field.

Response to metalaxyl

Agar assay. From each sampled field at each of the different sampling dates, two - four isolates were randomly selected from the four - eight isolates available per field and evaluated for growth on agar containing metalaxyl. The basal medium used in 1993 was rye agar without antibiotics, and in 1994, 2% V8 agar was used. Initially, colony growth on media containing 0, 5, and 30 or 50 μg/ml a.i. metalaxyl (Ridomil 25 WP) was measured; in all

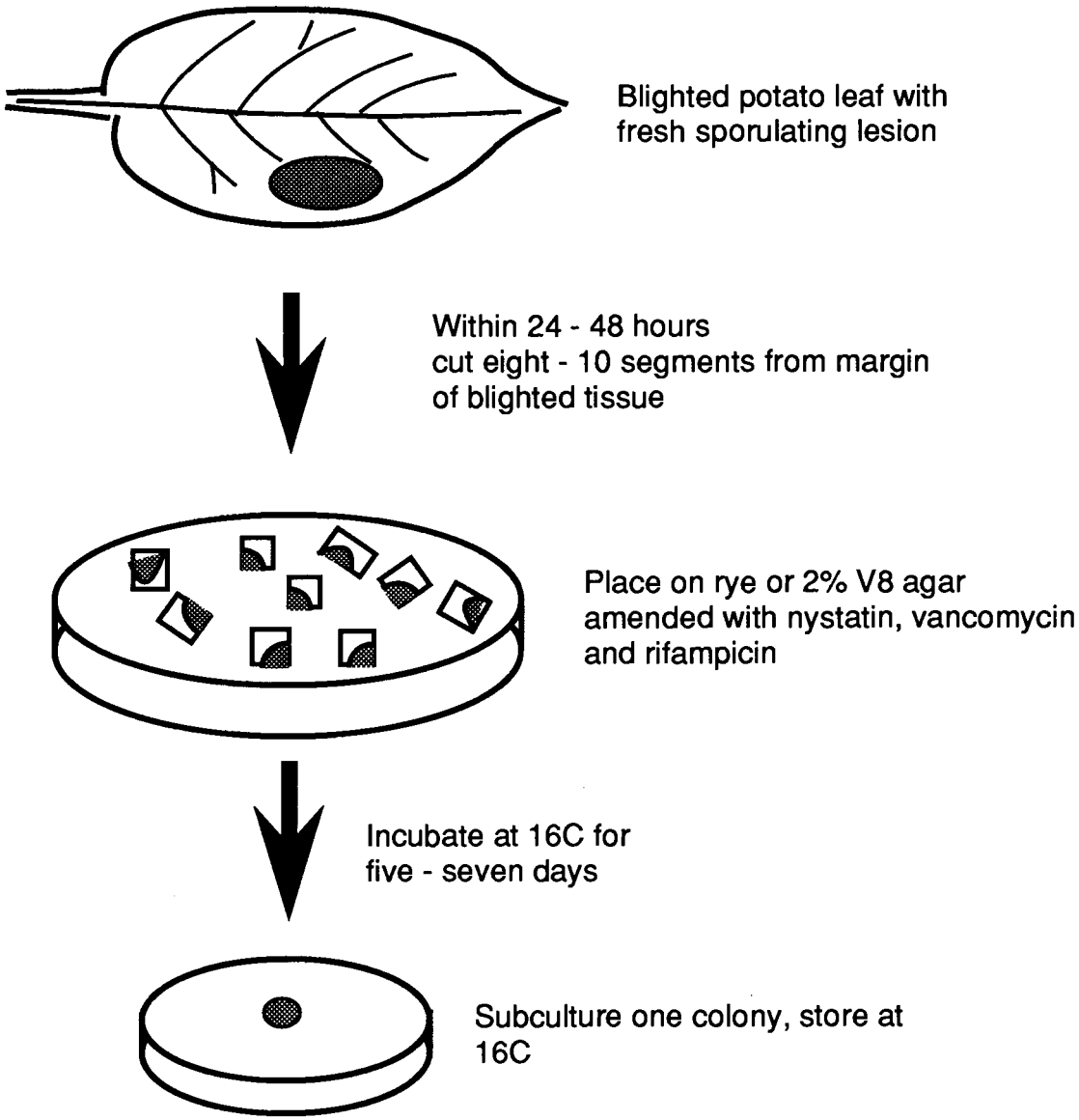


Figure 3. Pathogen isolation from plant tissue.

subsequent experiments, growth on 0 and 50 μg/ml was used to assess whether an isolate was sensitive (MS) or insensitive (MI) (Appendix 1). Colonies were initiated from 0.5 cm-diameter mycelial plugs taken from 14-28 day old cultures growing on rye agar or 2% V8 agar. Colony diameter was measured after 10-14 days of incubation at 20 C from two replicate plates by taking three measurements per plate. Initially, up to four plates per isolate were used, but the variation between plates was low enough to warrant using only two plates. Isolates with previously determined responses to this assay method were included: CG 96 (MS) and CG 215 (MI) (provided by C. Steden, Ciba, Basel), B.C. 4.1 (MS), B.C. 5.2 (MS) and B.C. 7.1 (MI) (provided by S. B. Goodwin, Cornell University, Ithaca). In most trials, CG 96 and CG 215 were included as standards. Isolates were considered to be insensitive if the ratio of growth at 50 μg/ml:0 μg/ml exceeded 0.3 i.e. if isolates grew at 30% of the controls in the presence of 50 μg/ml metalaxyl (Davidse et al 1981). In experiments where isolates grew poorly or the testers were inconsistent, the trials were repeated.

Leaf disc assay. Colonies of nine *P. infestans* isolates, including the two tester strains (CG96 and CG215), were grown on 2% V8 agar without antibiotics for two - four weeks at 16 °C in the dark. Each colony was then flooded with five -10 ml of sterile distilled water and gently agitated to release sporangia. The sporangial concentration was adjusted to 1-2 x 10^4/ml (average of four counts in a haemocytometer). Potato, cultivar Norchip, leaf discs (15 mm diameter) were taken from fully expanded leaves from plants grown in a room provided with supplemental halogen lighting (16 hr photoperiod). The leaf discs were misted with sterile distilled water and floated adaxial side up in petri dishes containing 20 ml of either sterile distilled water or 50 μg/ml metalaxyl (Ridomil 25 WP). The sporangial suspension (a 30 μl droplet) was placed on each leaf disc; there were eight discs per dish and two dishes per isolate. The dishes were incubated

at 16 °C and a 12 hour photoperiod until the control discs were completely colonized (usually seven - nine days). The percent area of the leaf disc covered with sporangia was then determined. The isolate was considered to be sensitive if the lesioned area was slightly necrotic, with no evidence of sporangia formation (Dowley and O'Sullivan, 1985). The experiment was conducted twice.

Mating type determination

Tester isolates of known mating type, which included WE 9 (A1) and B.C. 5.2. (A2) (provided by W. E. Fry, Cornell University, Ithaca) and B.C. 8.1.1. and B.C. 8.1.8. (both A2) collected in this study were used. Unknown field isolates (an average of two - four per sampled field, and in most cases the same isolates as those used in the metalaxyl test) were paired against each of the A1 and A2 tester isolate. Pairings were made on either agar-coated microscope slides or in 60 x 15 mm petri dishes containing 20% clarified V8 agar or 2% V8 agar (Galindo and Gallegly, 1960). Mycelial plugs (0.5 cm diameter) were placed one cm apart and the slides or dishes were incubated within a sealed container at 20 °C in the dark for two - three weeks. In some pairings, it was necessary to place the unknown isolate on the agar eight -10 days prior to the tester due to its slow growth. The presence or absence of oospores was recorded by viewing merged colonies directly with an inverted microscope (Figure 4). A zone of inhibition was often observed on plates with two identical mating types. Oospore formation in colonies paired against one of the tester strains denoted the opposite mating type in the unknown. In some isolates, oospore formation was observed against both of the tester strains and these isolates were often found

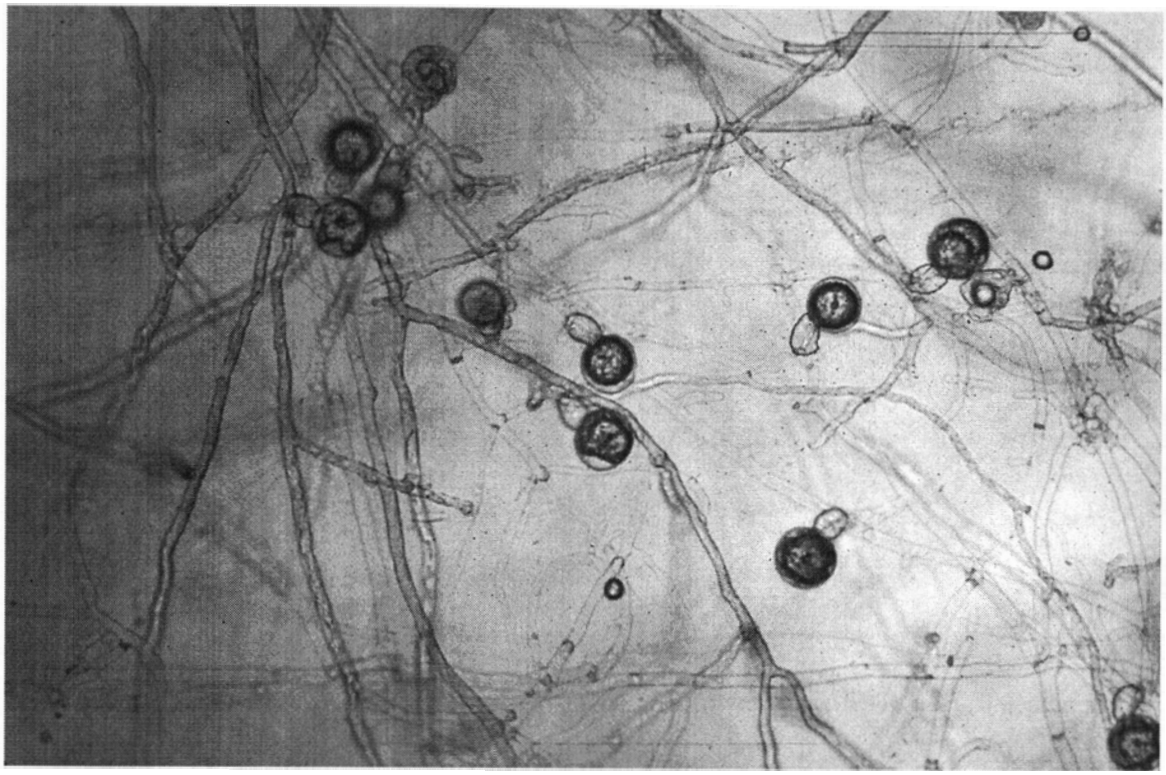


Figure 4. *Phytophthora infestans* oospores on 20% clarified V8 agar.

to produce oospores when selfed. Attempts to initiate colonies from single sporangia or a hyphal tip (Fyfe and Shaw 1992), in order to determine whether the isolates were an intimate mixture of both mating types or self-fertile, were unsuccessful; therefore, these isolates were categorized as being of mixed composition.

Oospore formation *in vivo*

Additional diseased leaves were collected from fields in B.C. and N.B. where leaf samples had yielded colonies of mixed composition or contained both mating types (Table 2, see Results section). Small pieces 0.5 cm^2 of the lesioned area from three - five leaves per field were fixed and cleared in 95% ethanol : glacial acetic acid (1:1) for four - five days, transferred to fresh fixative, and stored at room temperature. The cleared tissues were examined under phase contrast in the microscope to determine if oospores were present.

Survival of isolates under field conditions

Four isolates of *P. infestans*, collected during the 1993 growing season from the Fraser Valley of B.C. and which represented A1 MS, A1 MI, A2 MS and A2 MI, were grown on rye agar for 10 days. Potato cultivar Norchip tubers were dipped in 95% ethanol and a core (seven mm diameter) was removed and a five mm diameter mycelial plug (one isolate per tuber) was placed inside the cavity and the potato plug was replaced. The tubers were wrapped in moist paper towel and incubated in the dark at 18 C for one week. A few tubers were cut open to ensure that growth of *P. infestans* had occurred. The inoculated tubers were placed individually in nylon mesh bags which were filled with soil and buried to a depth of 10 cm in a field in Langley, B.C. which had not been planted to potatoes in 1993. For each isolate, there were six replicate tubers for each of

three sampling dates, and the tubers were randomized prior to burial (in December, 1993). At each sampling date, the tubers were recovered and isolation of the pathogen was attempted following surface sterilization in sodium hypochlorite (0.625%) and plating tuber pieces onto rye agar containing antibiotics.

Growth and sporulation of isolates *in vivo*

Eight isolates of *P. infestans* which represented A1 MS, A1 MI, A2 MS, and A2 MI were compared for rate and degree of colonization of potato leaf tissues. Colonies were grown on 2% V8 agar for two - four weeks and a sporangial suspension was prepared (1-2 x 10^4/ml). A 20 µl drop of inoculum was placed onto fully expanded leaflets of potato cultivar Norchip which were supported on the surface of water agar in 100 x 15 mm petri dishes. The dishes (four - six replications per isolate) were sealed with parafilm and incubated at 16° C for seven days, at which time the lesioned areas were traced onto clear acetate film and measured. The leaves were then placed in a test-tube containing 4 ml of water with a drop of Tween 20 and shaken vigorously and the number of sporangia was determined with a haemocytometer. The data were expressed as numbers of sporangia (average of four counts) per mm^2 of lesioned area. The experiment was conducted twice for each isolate tested.

RESULTS

Recovery of *P. infestans* from potato tissues

The pathogen was not always readily isolated from the leaf samples collected. It was intended that an average of 12-16 individual plants would be sampled per field, with six - eight tissue pieces plated per plant, to yield a minimum of four - eight isolates per field. However, not all of the fields sampled had active lesions from which *P. infestans* could be recovered; furthermore, many of the colonies which developed were contaminated and could not be subcultured. It was usually necessary to subculture twice to obtain contaminant-free colonies. On average, the recovery rate of *P. infestans* from lesions was 50%, yielding about two - three isolates per field (Table 1). The pathogen was recovered from 26 potato cultivars during two years of sampling (Table 1).

In 1993, 562 isolates were obtained from 80 different fields, plus one tomato late blight sample, at up to three different times throughout the growing season in B C., with each field representing a different location and cultivar. In 1994, 100 isolates were obtained from diseased leaves collected from 18 different fields plus two tomato gardens within B. C., and 75 isolates were obtained from 18 different fields in N.B. (Table 1). The difference in sampling frequency between 1993 and 1994 reflects the lower incidence of late blight in B.C. during 1994 due to hot and dry weather conditions, and the higher incidence of late blight in N.B. in 1994.

Response to metalaxyl

The *in vitro* agar assay measuring growth of *P. infestans* on 50 μg/ml metalaxyl relative to the unamended control provided a clear differentiation

Table 1. Recovery of isolates of *Phytophthora infestans* during 1993-1994 with regard to fields, potato cultivars, and time of season.

Year and geographic location	No. of different fields from which isolates were recovered	Month samples were collected	Potato cultivars from which isolates were obtained	Total number of isolates recovered
1993				
Alberta	7	July, Sept.	Norchip, Russet Burbank	8
British Columbia				
Fraser Valley	53	June, July, Aug., Sept., Dec. (storage)	Epicure, Eramosa, Krantz, Norchip, Norland, Red Lasoda, Russet Norkotah, Russet Burbank, Shepody, Sunrise, Warba, Yukon Gold	472
Okanagan Valley	7	Aug., Sept.	Nooksack, Russet Burbank, Russet Norkotah, Shepody	28
Pemberton Valley	10	July, Aug.	Chieftain, Pontiac, Ranger Russet, Russet Burbank, Russet Norkotah, White Rose	24
Vancouver Island	10	Aug.	Epicure, Nooksack, Red Lasoda, Shepody, Russet Burbank, Russet Norkotah, Yukon Gold	38

Table 1 (cont.)

Year and geographic location	No. of different fields from which isolates were recovered	Month samples were collected	Potato cultivars from which isolates were obtained	Total number of isolates recovered
Manitoba	4	Sept.	Russet Burbank	5
New Brunswick	3	Aug.	Atlantic, Russet Norkotah, Superior	7
1994				
Alberta	7	Sept.	All Blue, Banana, Russet Burbank	10
British Columbia				
Fraser Valley	14	June, Aug., Sept.	Eramosa, Hilite Russet, Nooksack, Norchip, Russet Burbank, Sunrise, Warba	92
Pemberton Valley	3	Sept.	Red Lasoda, Russet Burbank	5
Vancouver Island	1	Sept.	Russet Burbank	3
Manitoba	2	Aug.	Russet Burbank	4
New Brunswick	18	July, Aug.	A.C. Chaleur, Chieftain, Frontier Russet, Norris, Ranger Russet, Russet Burbank, Russet Norkotah, Shepody, Superior	75
Prince Edward Island	1	Aug.	Hilite Russet	4

between known sensitive and insensitive isolates. The response of these tester isolates to the *in vitro* assay method (on rye agar) is illustrated in Fig. 5A. A cut-off ratio of 0.3 was subsequently used to categorize the isolates. When the agar assay was compared to the leaf disc assay, seven of nine isolates tested showed identical responses to metalaxyl (Appendix 2). The frequency distribution of response to metalaxyl by isolates from B.C. in 1993 was bimodal, with a cluster of isolates below 0.3 (MS) and a cluster at 0.9-1.0 (MI) (Figure 5B). 79% of the isolates collected from throughout the province were MI. The frequency distribution of isolates collected in 1994 from B.C. and N.B. is shown in Figure 6. About 96% of the isolates collected in 1994 from B. C. were MI and 94% of the N.B. isolates collected were MI. The geographic origins of MS and MI isolates collected from B.C. in 1993 are indicated in Figure 7. Most of the insensitive isolates were recovered from Cloverdale, and all of the regions sampled (with the exception of Chilliwack) had both MS and MI isolates present (Figure 7). When the samples were considered with regard to time of season when they were collected, the majority of isolates in 1993 were recovered during July, when late blight was most severe (Figure 8A). The frequency of recovery of MI isolates was higher than or equal to MS (Figure 8), irrespective of the time of collection during the 1993 and 1994 seasons.

Mating type determination

The geographic origins of A1 and A2 mating types and the cultivars from which they were isolated during 1993 in B.C. are illustrated in Figure 9. The region with the highest frequency of A2 was Cloverdale and, with the exception of the Pemberton Valley, all of the regions sampled had combinations of both A1

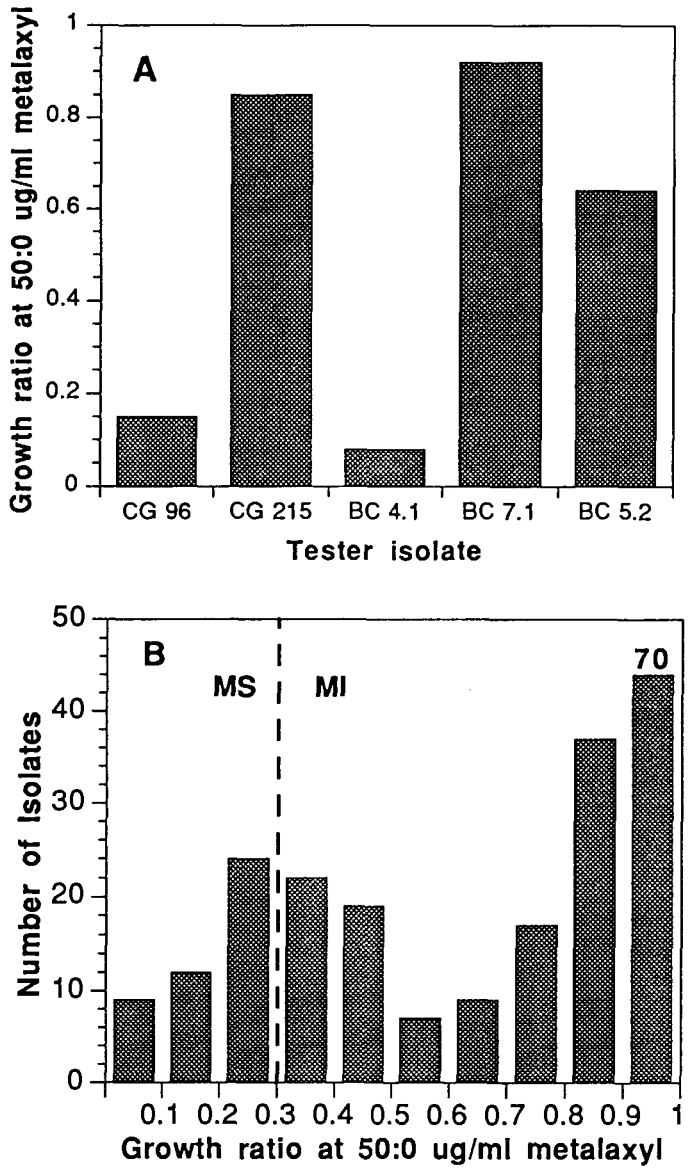


Figure 5. A) Response of isolates of *Phytophthora infestans* to metalaxyl using an *in vitro* agar assay measuring relative growth at 50 μg/ml metalaxyl and 0 μg/ml metalaxyl showing differentiation between sensitive (CG 96 and B.C. 4.1) and insensitive (CG 215, B.C. 5.2, B.C. 7.1) isolates. B) 1993 frequency distribution of isolates collected from British Columbia with different metalaxyl growth ratio (50:0 μg/ml) categories

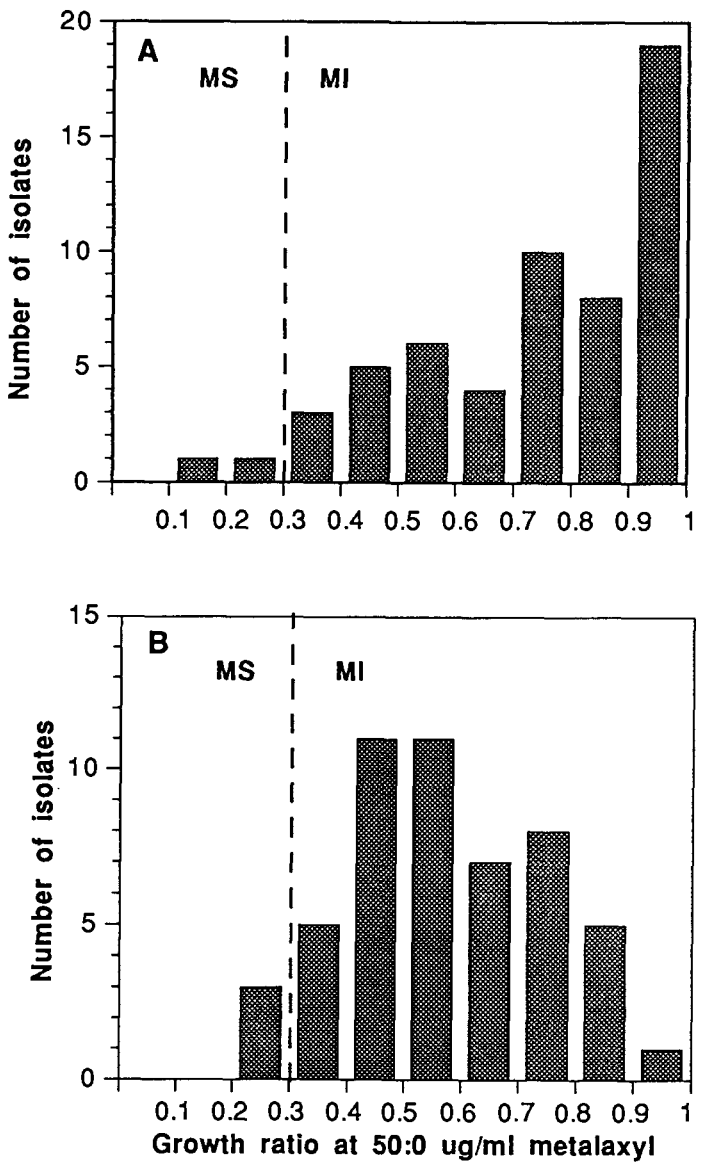


Figure 6. Frequency distribution of isolates of *Phytophthora infestans* in different metalaxyl growth ratio (50:0 µg/ml) categories. A) Isolates from British Columbia in 1994. B) Isolates from New Brunswick in 1994.

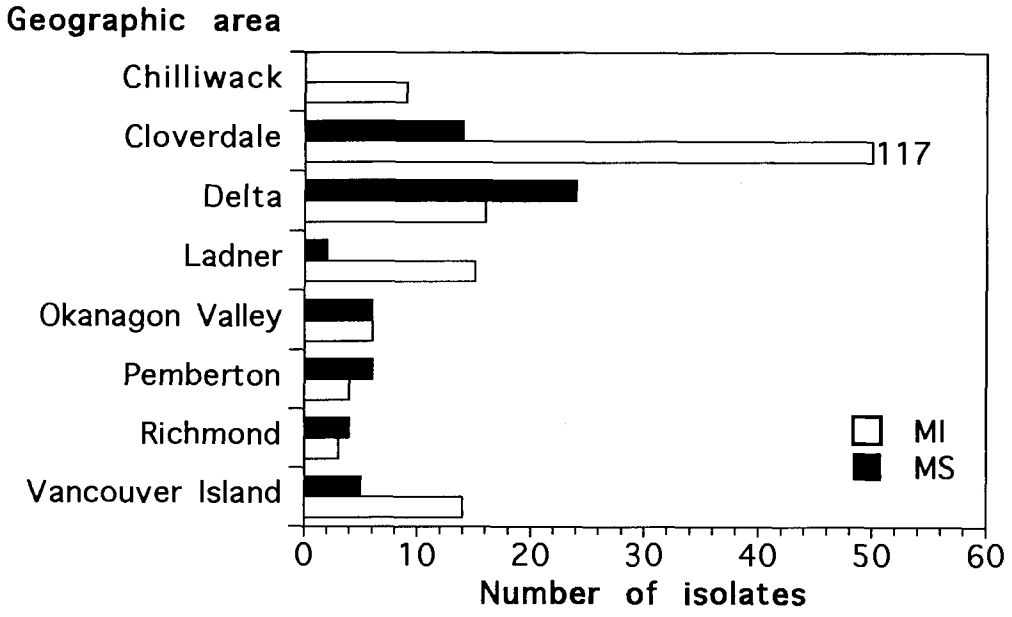


Figure 7. Geographic areas within British Columbia from which metalaxyl sensitive (MS) and metalaxyl insensitive (MI) isolates of *Phytophthora infestans* were recovered during 1993.

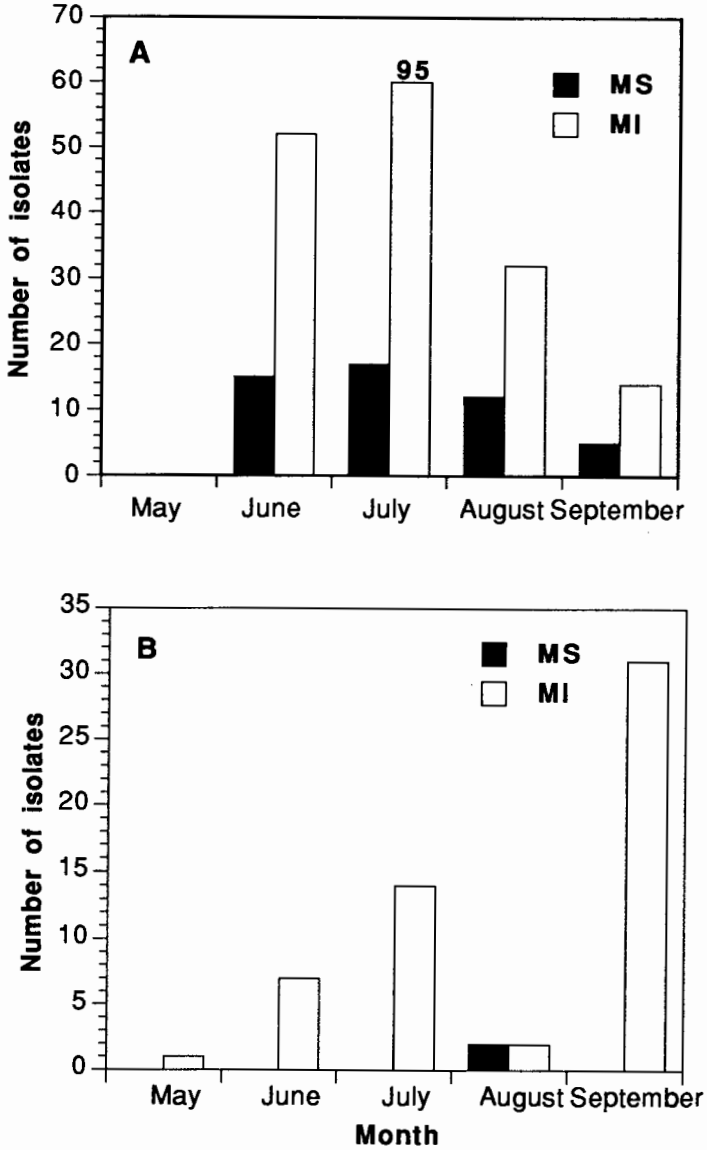


Figure 8. Number of metalaxyl sensitive (MS) and metalaxyl insensitive (MI) isolates of *Phytophthora infestans* recovered at monthly intervals in British Columbia A) 1993 growing season; B) 1994 growing season.

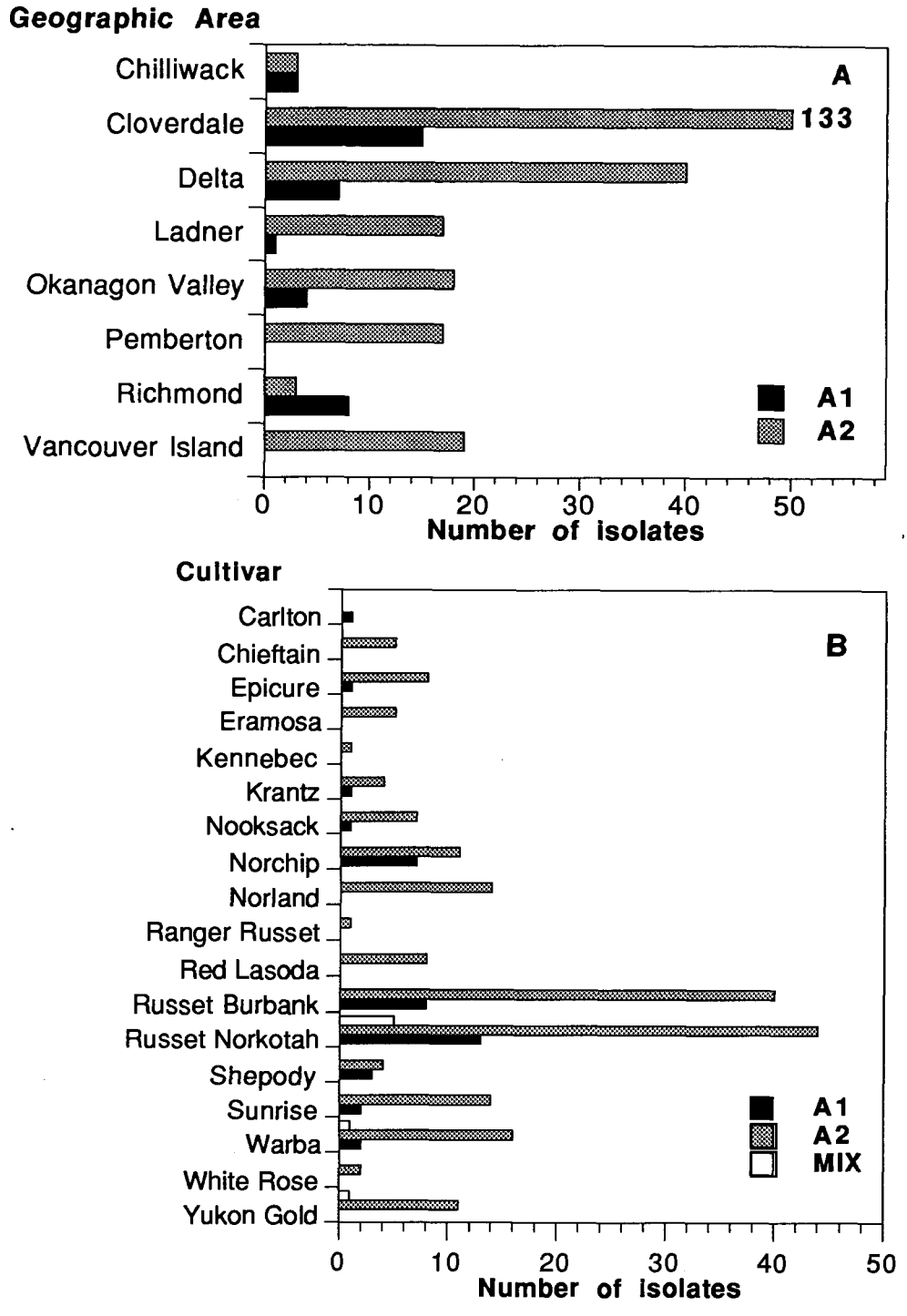


Figure 9. Recovery of A1 and A2 mating types of *Phytophthora infestans* from British Columbia during 1993. A) Geographic areas from which the two mating types were recovered. B) Potato cultivars from which the mating types were isolated.

and A2 (Figure 9A). Only one area (Richmond) had a higher frequency of A1 compared to A2. The A2 mating type was recovered from a wider range of cultivars than A1 (Figure 9B), with Russet Burbank and Russet Norkotah yielding the most isolates. Of the 288 isolates that were tested for mating type in 1993, 249 were A2 (Figure 10A). When the samples were considered with regard to the time of season when they were collected, the relative proportion of A2 was always higher than A1 throughout the 1993 growing season (Figure 10A). The total number of isolates collected was lower in 1994 than in 1993 (Figure 10A and B). In 1994, 35 of the 56 isolates tested for mating type were A2 (Figure 10B). The seasonal trend was similar to 1993, with the exception of July (Figure 10B). Most of the July isolates were collected in Richmond which had predominantly A1 isolates (Table 2).

Oospore formation *in vivo*

In 1994, three - five late blight lesions from potato leaves were sent in from one field in N. B. and one field in B. C. where isolates of mixed mating types had been identified earlier in the season. *P. infestans* sporangia were seen on the leaves at the margins of the lesions. When the leaves were cleared and examined for oospores, one double walled oospore was found from each field collection (Figure 11).

Characteristics of isolates collected throughout Canada

When the isolates originating from provinces other than B.C. were examined, all isolates collected in 1993 from Alberta, Manitoba, N. B., Nova Scotia, Ontario, Quebec and Prince Edward Island were of the A1 mating type

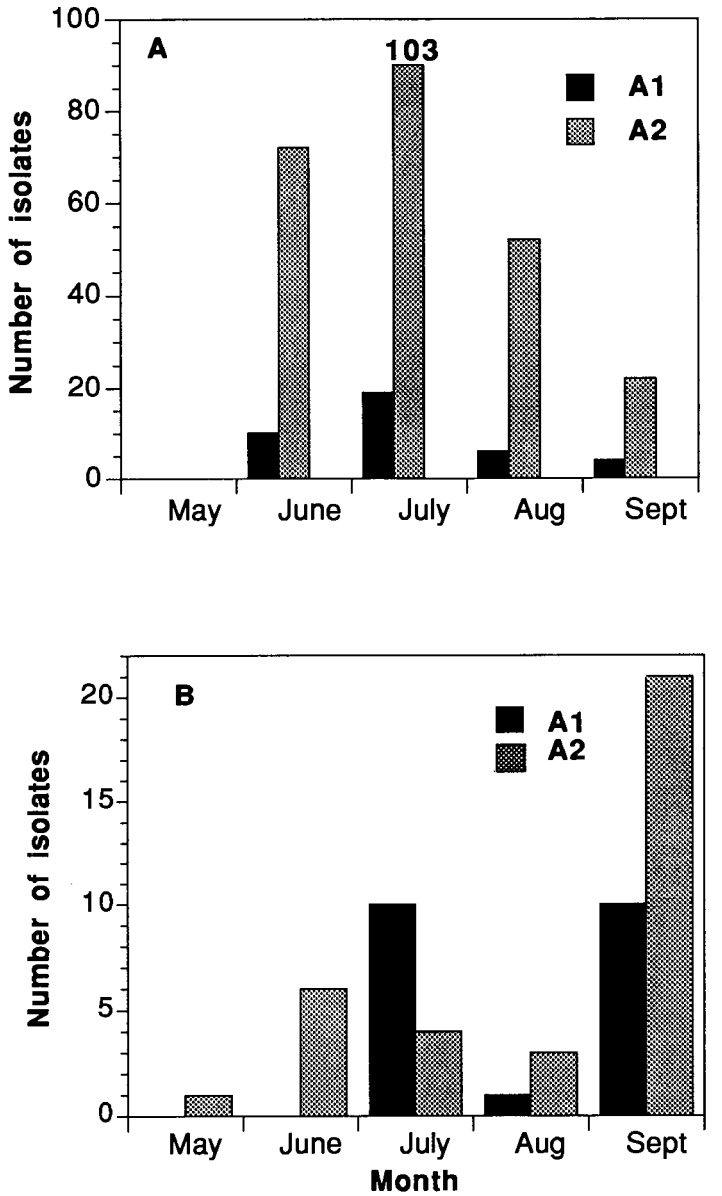


Figure 10. Number of A1 and A2 mating types of *Phytophthora infestans* recovered at monthly intervals in British Columbia A) 1993 growing season; B) 1994 growing season.

Table 2. Characterization of isolates of *Phytopthora infestans* from Canada during 1993-1994 with regard to mating type and response to metalaxyl *in vitro*[a].

Geographic origin of isolate[b]	Response to metalaxyl and mating type					
	Sensitive			Insensitive		
	A1	A2	Mix[c]	A1	A2	Mix[c]
1993						
Alberta -						
Brooks	--	--	--	1	--	--
Duchess	2	--	--	--	--	--
Lethbridge	1	--	--	--	--	--
S. Alberta	3	--	--	--	--	--
Taber	1	--	--	--	--	--
British Columbia -						
Chilliwack	--	--	--	2	5	--
Cloverdale	1	11	1	10	91	1
Delta	--	15	--	5	16	--
Ladner	--	2	--	1	12	--
North Vancouver	--	--	--	1	--	--
Okanagan	--	6	--	2	4	--
Pemberton	--	3	--	--	3	--
Richmond	3	--	--	2	1	--
Vancouver Island	--	2	1	--	11	2
Manitoba -						
Bruxelle	1	--	--	--	--	--
Plum Coulee	1	--	--	--	--	--

Table 2 (cont.)

Geographic origin of isolate[b]	Response to metalaxyl and mating type					
	Sensitive			Insensitive		
	A1	A2	Mix[c]	A1	A2	Mix[c]
Portage La Prairie	3	--	--	--	--	--
New Brunswick -						
Grand Falls	3	--	--	--	--	--
Haltland	3	--	--	--	--	--
Undine	1	--	--	--	--	--
Nova Scotia[b] -	20	--	--	--	--	--
Ontario[b] -	4	--	--	--	--	--
Prince Edward Island[b]	12	--	--	--	--	--
Quebec[b]	1	--	--	--	--	--
1994						
Alberta -						
Edmonton	6	--	--	1	--	--
Spruce Grove	6	--	--	2	--	--
British Columbia -						
Cloverdale	--	--	--	9	28	--
Vancouver Island	--	1	--	--	--	1
Delta	--	--	--	2	4	--
Pemberton	--	1	--	--	--	--
Richmond	--	--	--	8	--	--

Table 2 (cont.)

Geographic origin of isolate[b]	Response to metalaxyl and mating type					
	Sensitive			Insensitive		
	A1	A2	Mix[c]	A1	A2	Mix[c]
Manitoba -						
Portage La Prairie	3	--	--	--	--	--
New Brunswick -						
Bon Accord	--	--	--	--	4	--
Charleston	--	--	--	--	--	1
Drummond	--	--	--	3	1	3
Four Falls	--	--	--	1	--	3
Glassville	--	--	--	1	--	--
Grand Falls	--	--	--	1	--	--
Greenfield	--	1	--	--	--	4
Holmesville	--	1	--	--	2	2
Knoxford	--	--	--	1	1	1
Limestone	--	--	--	1	3	--
New Denmark	--	1	--	2	2	4
Upper Kent	--	--	--	--	1	2
Woodstock	1	--	--	--	2	--
Prince Edward Island	1	--	--	--	--	--

[a] In 1993 283 isolates were tested for both mating type and reaction to metalaxyl, in 1994 a total of 122 isolates were tested for both characteristics

[b]Isolates provided by G. White

[c]Both A1 and A2 mating types present in one sample.

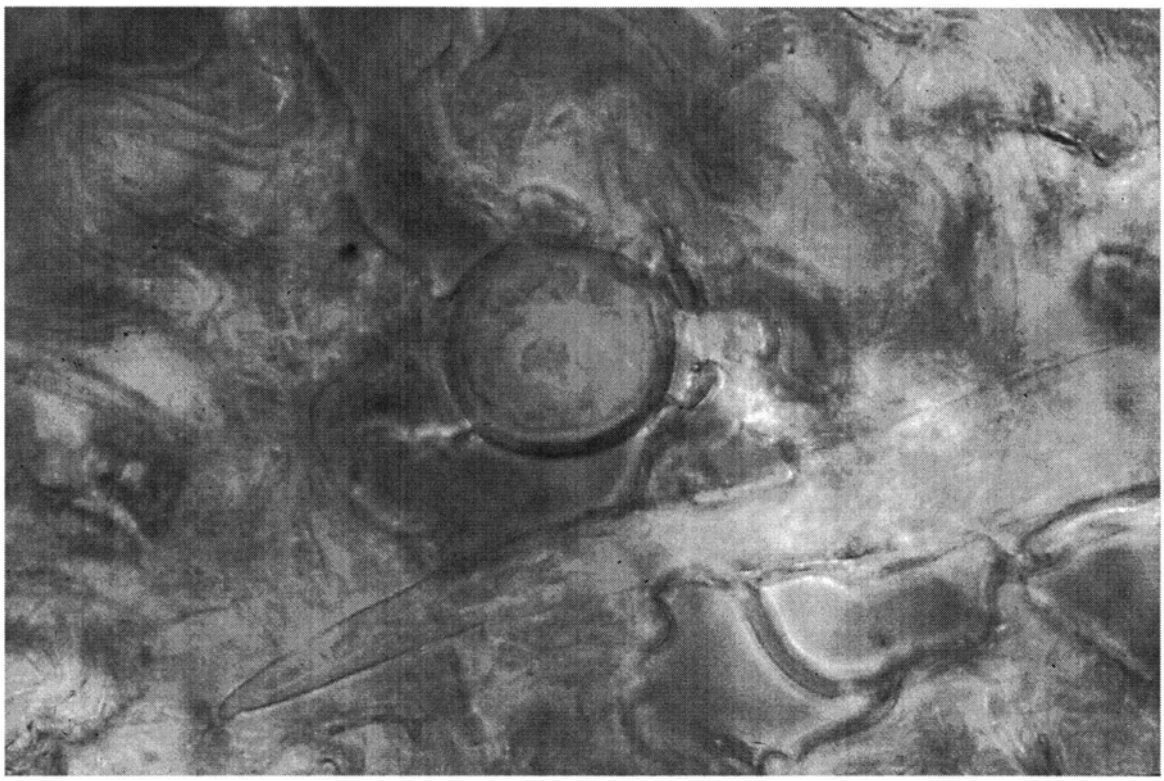


Figure 11. Oospore of *Phytophthora infestans* observed in potato leaf collected in New Brunswick, 1994.

and all isolates (except one from Brooks, Alberta) were found to be MS (Table 2). During 1994, A2 was found in N. B., where late blight was very severe. Both mating types were found in mixed cultures which originated from single lesions on individual leaves collected from different fields (Figure 12). A majority of the mixed cultures were insensitive to metalaxyl (Table 2). The cultivars from which A1, A2 and mixed cultures were recovered are illustrated in Figure 12. The highest proportion of isolates recovered from N.B. in 1994 were from mixed fields, in which both A1 and A2, or mixed isolates were found. When the samples were considered with regard to the time of season they were collected, the majority were recovered in July and were MI (Figure 13A) and of mixed mating type (Figure 13B). The proportion of A1 was higher than A2 in July (Figure 13B). The mean colony diameter of A2 strains collected from N.B. in 1994 was higher than that of A1, while the average diameters of MI and of MS strains were not significantly different (Table 3).

Characteristics of the population from B.C.

The mean colony diameters of A1 and A2 isolates were not significantly different in 1993; however the means were different in 1994 (Table 3). The mean diameters of B.C. isolates in the MS and MI categories were significantly different only in 1993 but the average diameter of isolates was lower in 1994 (Table 3). The colony growth of isolates collected in 1993 on rye agar was variable, with the A2 isolates displaying growth over a wider range than A1 isolates (Figure 14A and Table 3). Most of the A2 colonies grew between 3.0 to 6.0 cm in 10 days, but some reached diameters of up to 7.5 cm. The A2 isolates collected from B.C. in 1994 had a lower mean colony diameter but displayed a

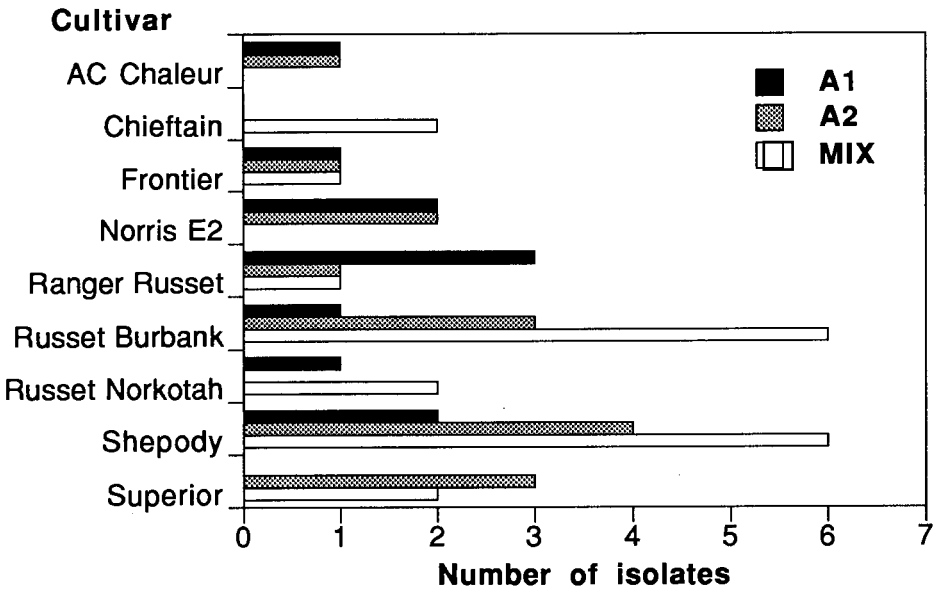


Figure 12. Recovery of A1 and A2 mating types, and mixed colonies, from different potato cultivars grown in New Brunswick during 1994.

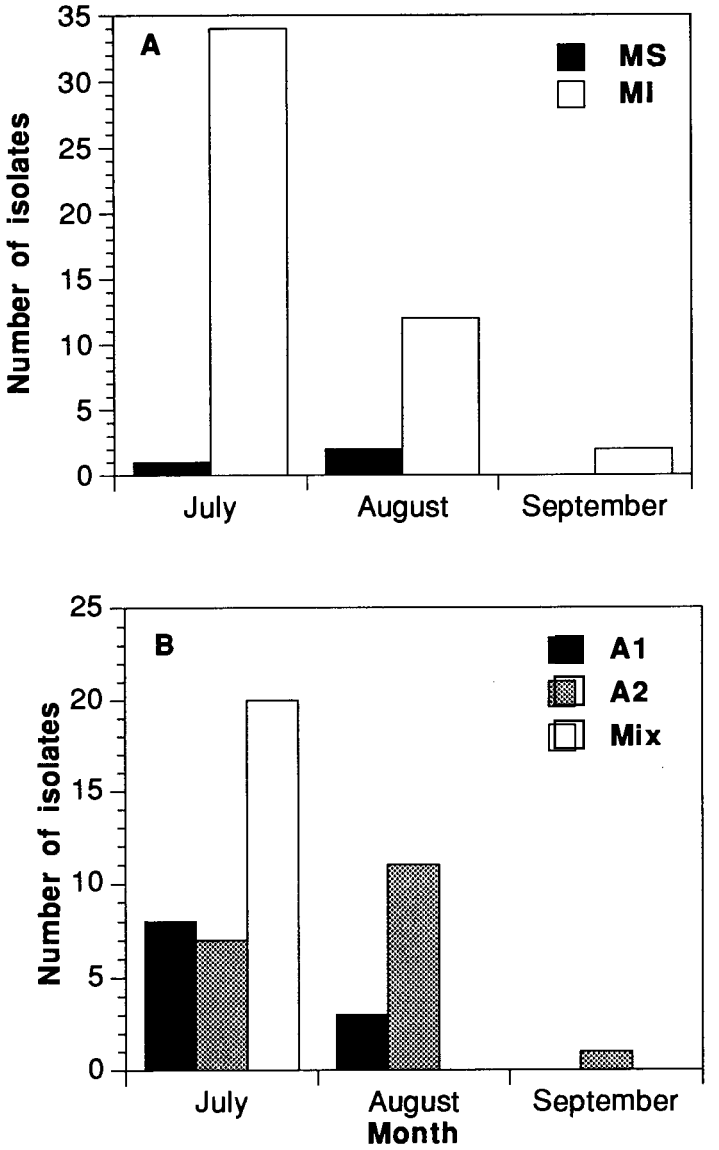


Figure 13. A) Numbers of metalaxyl sensitive (MS) and metalaxyl insensitive (MI) isolates of *Phytophthora infestans* recovered at monthly intervals during the 1994 growing season in New Brunswick B) Numbers of A1, A2 and mixed mating types recovered at monthly intervals during the 1994 growing season in N.B.

Table 3. Colony diameters of *Phytophthora infestans* strains grouped according to A1 or A2 mating type, sensitivity to metalaxyl, year and province[a]

category	mean	n	P value (t test)
British Columbia, 1993			
A1	44.29	24	
A2	43.43	158	0.8534
MS	37.22	48	
MI	45.87	163	0.0004 *
British Columbia, 1994			
A1	23.14	19	
A2	31.54	34	0.0214 *
MS	17.7	2	
MI	28.25	55	0.2978
New Brunswick, 1994			
A1	45.95	11	
A2	63.31	18	0.0063 *
MS	54.56	3	
MI	51.49	48	0.8821

[a]Growth was measured after 10 days of incubation at 20°C on rye agar in 1993 and on 2% V8 agar in 1994. Diameters are the average from the two replicate 0 µg /ml metalaxyl dishes used in metalaxyl sensitivity tests.

* Analysis with t test indicates that the probability of a greater value of t due to chance were * < 0.05. (SAS. 1988)

In 1993, of 288 isolates tested for mating type, 182 were tested for metalaxyl sensitivity on rye agar. From the 1994 collection; of 56 B.C. isolates tested for mating type, 53 were tested for metalaxyl sensitivity, and of 50 N.B. isolates tested for mating type 29 were tested for metalaxyl sensitivity.

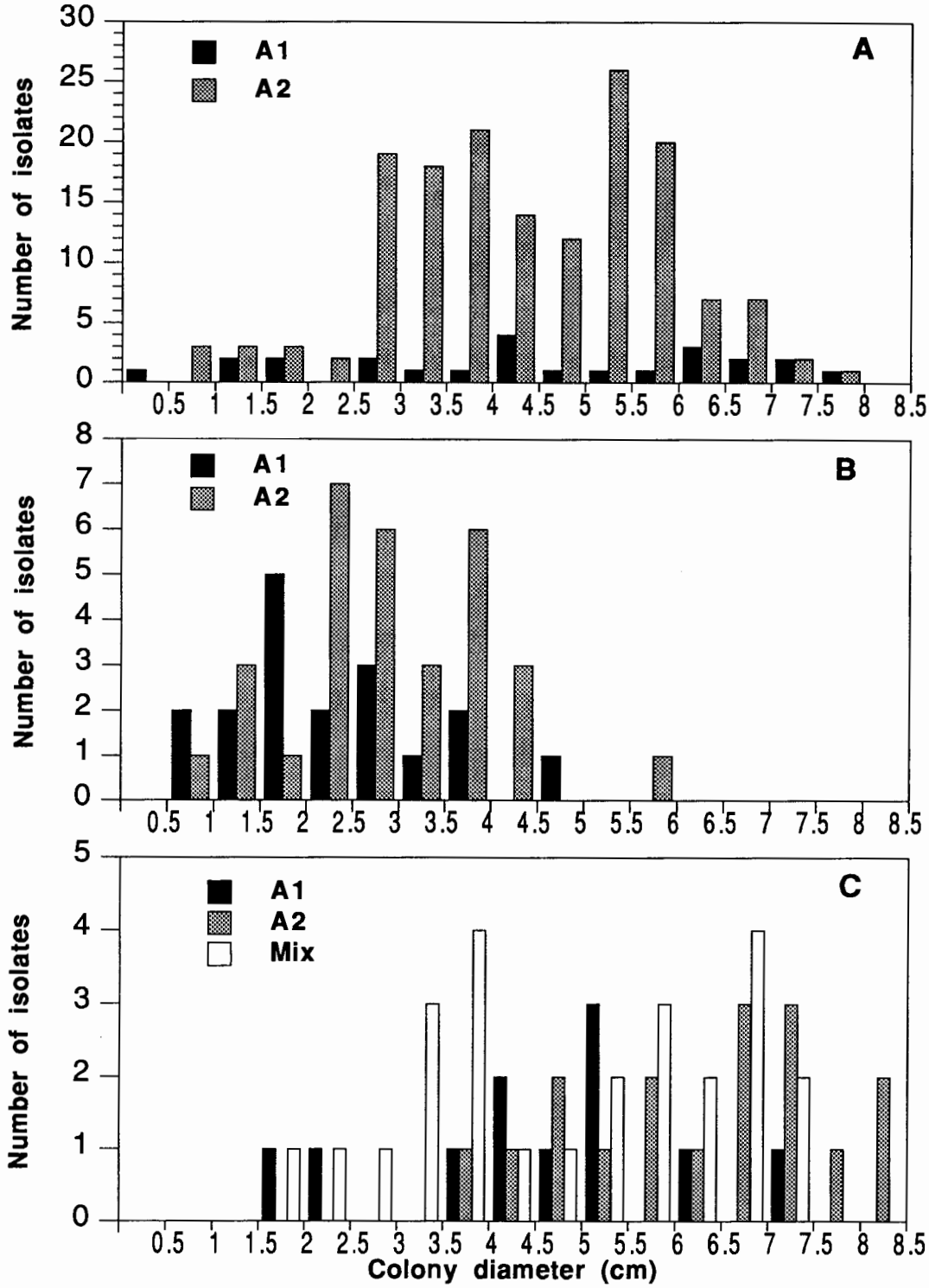


Figure 14. Colony diameters of isolates of A1 and A2 mating types of *Phytophthora infestans* grown on agar at 20 C for 10 days. A) Isolates collected from British Columbia during 1993 (rye agar). B) Isolates collected from B.C. during 1994 (2%V8 agar). C) Isolates collected from New Brunswick during 1994 (2% V8 agar).

similar range of growth (Figure 14B). In comparing the morphology of isolates of the A1 and A2 mating types of *P. infestans* in culture, there were no specific characteristics which were unique to either mating type. Some isolates had dense fluffy mycelium while others had thin patchy mycelium and linear growth was variable as noted in the ranges of the colony diameters (Figure 14A and B).

During the sample collection period, information was obtained on whether metalaxyl had been applied to individual fields prior to the time of sampling. Farmers in 1993 were hesitant to use Ridomil MZ due to the suspected insensitivity in *P. infestans*, thus only 12 of the fields sampled received Ridomil MZ application. The remaining 68 fields not receive Ridomil MZ. When the frequency of recovery of MS and MI isolates was compared with the use of metalaxyl during the same season, there were no significant differences between the different categories (Table 4). MI and MS isolates were found in fields in which metalaxyl had not been used and also in fields which had received with metalaxyl (Table 5). A comparison of mating type with metalaxyl response indicated that most of the isolates from B.C. in 1993 were A2 MI, followed by A2 MS, and a small proportion (four isolates) were A1 MS (Table 6).

Table 4. Frequency of recovery of metalaxyl-sensitive and metalaxyl-insensitive isolates of *Phytophthora infestans* from fields with or without metalaxyl application in the same season[a]

	Recovery of	
Metalaxyl applied	**Metalaxyl-sensitive**	**Metalaxyl-insensitive**
Yes	7	31
No	46	159

[a]Data from 72 fields in British Columbia during 1993.

Chi-square statistic 0.19, with 1 degree of freedom. The probability of a greater Chi-square due to chance was > 0.50. (Zar, 1984)

Table 5. Seasonal recovery of metalaxyl-insensitive isolates of *Phytophthora infestans* from fields with or without metalaxyl application in the same season

	Frequency of recovery of metalaxyl insensitive isolates from fields	
Sampling time	**With metalaxyl**	**Without metalaxyl**
June	0.80 (n=10)	0.73 (n=60)
July	0.81 (n=16)	0.83 (n=93)
August	0.78 (n=9)	0.71 (n=35)
September	1.00 (n=3)	0.69 (n=16)

Data from isolates collected in British Columbia during 1993.

Table 6. Relationship between mating type and response of isolates to metalaxyl[a]

	Metalaxyl response		
Mating type	**Sensitive**	**Insensitive**	**Total**
A1	4	23	27
A2	39	143	182
Total	43	166	209

[a]Summary of data for isolates tested for both mating type and metalaxyl sensitivity in 1993 originating from British Columbia.

Survival and fitness of isolates.

Isolates were successfully recovered from tubers after 28 and 72 days only. The tubers were thoroughly rotten and unrecoverable after 100 days of burial in the field (early April). The recovery of isolates from buried tubers after 72 days was comparable for A1 strains (2.5-4.0%) and for the A2 strains (6.0-11.0%). These were not significantly different (Appendix 3).

The results of the fitness test measuring sporangial formation and lesion size on potato leaves were inconclusive. The measurements of sporangial numbers/mm^2 of lesioned area for isolates representing A1 MS, A1 MI, A2 MS and A2 MI revealed a high degree of variability between replications and repetitions of the experiment. Therefore, no significant differences could be detected between the various isolate categories (Appendix 4).

DISCUSSION

The results from this two-year study provide information on the mating types and metalaxyl sensitivity of populations of *P. infestans* on potatoes in Canada, similar to studies conducted in the United States (Deahl et al 1993, Fry et al 1993,Goodwin et al 1994, Goodwin et al 1995) and elsewhere (Dowley and O'Sullivan 1985, Drenth et al, 1994a, Koh et al, 1994, Mosa et al, 1989, Shattock et al 1990,Sujkowski et al 1994). The results also illustrate some of the changes that are occurring in the populations from one year to the next. Although a widespread and systematic sampling was not conducted in fields representing all Canadian provinces, the results show some clear trends.

The widespread presence of the A2 mating type was confirmed in B.C. in 1993 following its initial discovery in 1989 (Deahl et al 1991). Although isolates of the A2 mating type were not detected from any of the other Canadian provinces sampled in 1993, they were subsequently found in N. B. in 1994. The A2 mating type has now also been reported from four additional provinces: Alberta, Manitoba, Ontario and Quebec (H. W. Platt, personal communication). The prevalence of the A2 mating type within B.C. and its appearance in other provinces within Canada was not unexpected, given the rapid migration patterns elsewhere in the world (Fry et al, 1993, Spielman et al, 1991). Many potato production fields in N. B. are also located in close proximity to fields in Maine, USA where the A2 mating type has been reported (Goodwin and Fry, 1994), and movement of seed potatoes occurs between the two countries.

The widespread occurrence of the A2 mating type in most potato production fields in B.C. is likely the outcome of dissemination of diseased tubers and spread of inoculum from adjoining fields, cull piles, or volunteer potatoes over several growing seasons. The Cloverdale area, from which a majority of the

samples originated, represents an area of about 30 km^2, making it relatively easy for airborne sporangia to spread from one field to another (Fry et al, 1993). Although the first report of the A2 strain was in 1989 (Deahl et al, 1991), it is not clear how long it has been present in B.C., nor all of the environmental conditions or cultural practices that may have contributed to its spread. It is unclear how the A2 mating type was introduced into the Pemberton Valley, because all potato seed planted in the valley originates from tissue culture. It is postulated that the A2 mating type may have been first introduced into B.C. through a shipment of diseased tomato seedlings in 1986 (D. J. Ormrod, personal communication). Regardless of the initial inoculum source, spread has been rapid, similar to that in other regions of the world (Fry et al, 1993, Spielman et al, 1991).

Despite the predominant occurrence of isolates of the A2 mating type in B.C., a proportion of the A1 mating type was recovered consistently in 1993 (16%) and 1994 (38%). The large sample size (288 isolates in 1993) over four relatively small geographic areas within British Columbia would have enhanced the probability of recovery of this strain which occurs at a lower frequency. In many regions where sampling for late blight has been conducted, the A2 mating type appears to have displaced the A1 mating type (Fry et al 1993, Goodwin et al, 1994, Grinberger et al, 1989, Koh et al, 1994, Mosa et al, 1989, Spielman et al, 1991, Therrien et al, 1993). In other regions, including some Canadian provinces, the A2 mating type has not yet been detected and the populations currently are still comprised of the A1 mating type (Andrivon et al, 1994, Deahl et al, 1993).

The 1993 and 1994 data show a *P. infestans* population in B.C. with isolates that have overcome the resistance of the cultivars Nooksack and Eramosa when grown under B.C. conditions (Platt and McRae, 1990, Stevenson,

1993). The A2 mating type was collected from both of these cultivars. This observation suggests that the A2 mating type is as capable of overcoming R-gene resistance as the population representing the A1 mating type, however; virulence testing of the A2 isolates is required to test this. Cultivar evaluations for resistance to late blight should take into consideration the different levels of complexity of the pathogen populations in different potato growing regions in Canada and elsewhere.

Laboratory studies which compared isolates of the A1 and A2 mating types and varying metalaxyl sensitivities for rates of colonization of leaf tissues and extent of sporulation did not reveal any differences, due to considerable variation within and between strains of one mating type. Since the genotypic background of these strains was unknown, it would be difficult to attribute any fitness characteristics to a particular mating type unless the isolates were determined to be genetically similar. Deahl et al (1991) were unable to detect any differences in virulence of the A1 and A2 mating types. Metalaxyl insensitivity does not appear to be linked to one particular mating type as observed in other studies (Deahl et al, 1991, Spielman et al, 1991). A comparison of *in vitro* colony growth rates showed a difference in the means of the MS and MI samples collected in B.C. during 1993, with the MI isolates having a greater mean growth rate. However, differences in growth and aggressiveness between metalaxyl sensitive and insensitive strains have been reported (Bashan et al, 1989, Dowley, 1987, Kadish et al, 1990, Kadish and Cohen, 1992).

A comparison of *in vitro* colony growth rates of isolates of A1 and A2 mating types in this study revealed significant differences in samples from B.C. and from N.B. during 1994 with the A2 isolates growing faster than the A1 isolates. A2 isolates collected from N. B. in 1994 were shown to be genotype U.S.-8 (Goodwin and Fry, 1994) and the A1 may represent U.S.-1 (Goodwin et

al, 1994). However, the genotype of the A2MS and A1MI isolates collected from N.B. in 1994 remains unclear.

The seasonal changes in the prevalence of specific strains of the late blight pathogen within a small geographic area have not been previously studied. By conducting early, mid, and late-season sampling of the same potato fields in B.C. during 1993 and 1994, the results revealed that a high proportion of isolates found early in the season (June) were of the A2 mating type. This early-season occurrence may be due to an overwintering component of the fungus under B.C. conditions, or to the reintroduction of the pathogen on infected seed tubers.

Isolates of both the A1 and A2 mating types were recovered from the same fields, and in some cases from the same plants or leaves, in both B.C. and N.B. in 1994. In many regions where late blight sampling studies have been conducted following the appearance of the A2 mating type, it has been reported that fields predominantly contained isolates of one mating type, either the A2 (Grinberger et al, 1989, Therrien et al 1993) or A1 (Andrivon et al 1994, Deahl et al, 1993). However, both A1 and A2 mating types have been reported from the same field in Switzerland (Hohl and Iselin, 1984), Korea and Japan (Koh et al, 1994, Mosa et al, 1989), England (Shattock et al, 1990, Shaw, 1987), and Poland (Sujkowski et al, 1994). A large proportion of the isolates originating from N.B. in 1994 were categorized as "mixed" due to oospore formation with both tester isolates. Since colony transfers were made using mycelial plugs in this study and not from single sporangia, mycelium from lesions which contained both mating types were not distinguished during isolation. Single lesions have rarely been found to contain mixtures of two isolates, and self-fertile cultures from either mixtures of A1 and A2 hyphae or from a single genotype are reported to occur rarely (Fyfe and Shaw, 1992, Mosa et al, 1989, Shattock et al, 1990,

Shaw, 1987). Self-fertile isolates recovered from potato foliage in England and Wales were proven to be intimate mixtures of A1 and A2 isolates, (Fyfe and Shaw, 1992, Shattock et al, 1990). In most of the mixed cultures, oospore formation was observed without any pairings with tester isolates, or where pairings were done, oospores formed against both A1 and A2 testers. Although *P. infestans* is reported to form oospores in self-fertile cultures (Shaw, 1987), this phenomenon is unlikely to explain our observations. In all of the fields in B.C. and N.B. in which mixed colonies were detected, lesions that yielded individual A1 and A2 mating type were also present on other plants. The data collected in this study may represent a field in an epidemic situation with several lesions occurring per leaf. Neighbouring blight infected fields in Maine may have provided the air-borne inoculum, therefore these data may be reflecting the initial introduction of A2 into an area. The shifts in mating type composition of the population in N. B. from A1 domination in the spring to almost equal proportions in the fall, also may indicate initial introduction of the A2 mating type.

The occurrence of both mating types on a single leaf suggested the possibility for oospore production *in planta*. Examination of numerous leaf samples originating from fields where mixed populations were found in B.C. and N.B. during 1994 revealed two samples (one from each province) to contain an oospore. This appears to represent the first report of oospore production by the late blight pathogen in naturally infected leaves from North America. Oospore production has been reported from other regions following artificial inoculation with both mating types (Deahl et al, 1991, Frinking et al, 1987, Grinberger et al, 1989, Pittis and Shattock, 1994). Although the frequency of oospore formation in diseased leaves in this study was extremely low, it suggests the possibility for sexual recombination and increased genetic variation in populations of this pathogen in B.C. and N.B. A report describing four unique genotypes of *P.*

infestans originating from B.C. in 1991-1992, for example (Goodwin et al, 1995), may implicate sexual recombination as one of the possible origins of these genotypes. Therefore, it is conceivable that oospore formation, as observed in this study, could (or has) given rise to an increase in unique genotypes. Additional molecular studies similar to those reported by Goodwin et al (1995) are needed to confirm the extent to which these genotypes are distributed. Increased genetic diversity in other regions of the world has been attributed to sexual recombination (Drenth et al, 1994a, Goodwin et al, 1992, Matuszak et al, 1994, Sujkowski et al, 1994, Therrien et al, 1993). It is still undetermined, however, whether the increased variation from sexual populations will have an impact on fitness and virulence of strains of *P. infestans* (Tooley et al, 1986). Furthermore, the genetic structure of populations appears to change dramatically from one year to the next (Drenth et al, 1994a, Goodwin et al, 1995).

The response of isolates of *P. infestans* to metalaxyl was tested using an *in vitro* agar assay which was found to provide data that correlated with the *in vivo* leaf disc assay. Matuszak et al (1994) also observed a good correlation between the agar assay and the leaf disc assay, with the former being easier to conduct and evaluate. However, the *in vivo* leaf disc assay provides more precise indications of metalaxyl sensitivity (Sozzi and Staub, 1987) and it is conceivable that the agar assay may overestimate metalaxyl insensitivity as there are no parameters for measuring intermediate sensitivity. A screening of 350 isolates of *P. infestans* from throughout Canada to metalaxyl showed that all isolates with the exception of those from B.C. and one isolate from Alberta in 1993 were MS. In B.C., a high proportion (73%) of isolates were MI. In 1994, 88% of the isolates tested from N.B. were MI. MI isolates were also collected from several other Canadian provinces including Ontario and Quebec (H. W. Platt, personal communication). Metalaxyl insensitivity was associated with both

mating types in B.C., but a higher association was seen with the A2 mating type. However, metalaxyl sensitive A1 and A2 strains were also recovered at a moderate frequency (30%). Studies on the distribution of MI in other regions have shown that populations may be almost completely insensitive (Deahl et al, 1993) or comprise a mixture (Andrivon et al, 1994, Koh et al, 1994, Matuszak et al, 1994, Shattock et al, 1990, Therrien et al, 1993).

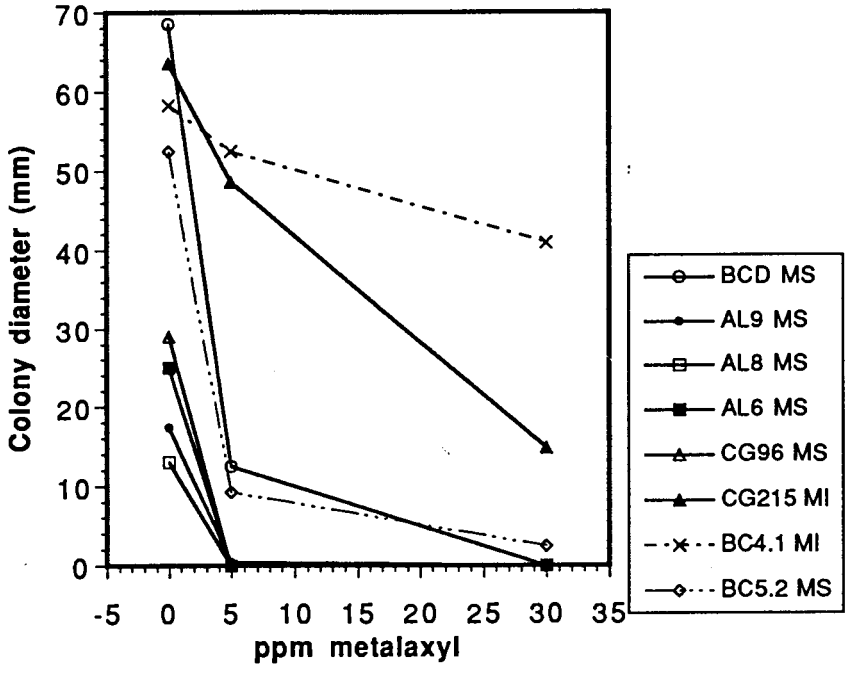
MS and MI strains were recovered from all sampled areas in B.C., and both were found together in six fields. The seasonal distribution of MS and MI isolates in B.C. indicated that MI isolates appeared early in the season (June), in some cases in fields that had not been treated with the fungicide. This observation suggests that MI isolates may be introduced early in the season on infected seed tubers or that a proportion of isolates can overwinter under B.C. conditions. The proportion of insensitive to sensitive isolates was comparable whether or not metalaxyl had been applied. This lack of correlation suggests that selection for metalaxyl insensitivity may have previously occurred in the B.C. population and that detectable shifts are not seen during one growing season. Cohen and Samoucha (1989) similarly observed that with a high initial frequency of metalaxyl insensitivity in a population, isolates with insensitivity are maintained regardless of fungicide application.

Metalaxyl insensitivity may have developed in the population in B.C. between 1985 and 1989. Reports of inadequate disease control following fungicide applications in 1990 suggest that MI strains have been present for several years (D. Ormrod, personal communication). The frequency distribution of isolates from B.C. in relation to growth on 50 μg/ml metalaxyl was bimodal, with a peak for each of MS and MI isolates. A similar response was observed in a population of isolates from Mexico, in a region where sexual recombination was occurring which contributed to the increased variation (Matuszak et al,

1994). The population from B.C. showed a similarly large range of variation in response to metalaxyl, although it is unknown whether this is an outcome of sexual recombination. The extent to which this variability may persist over successive growing seasons is also unknown. Less variability in growth response to metalaxyl was present in the population from N.B. in 1994, in which the A1 mating type and mixed populations were detected for the first time in 1994. Given sufficient time, this population may increase in variation similar to that observed in B.C. and Mexico. A comparison of colony growth rates of isolates which were sensitive and insensitive to metalaxyl showed that in 1993, metalaxyl insensitive isolates grew faster. Metalaxyl insensitive isolates have been reported to display reduced sporulation and survival (Dowley 1987, Kadish and Cohen, 1992) or conversely, be better able to infect and sporulate (Bashan et al 1989, Kadish et al, 1990).

It is not clear whether a decrease of the use of metalaxyl would correspond with a decline in the occurrence of metalaxyl MI strains in B.C. Such a phenomenon has been reported in areas where only one mating type was present and the population was presumably uniform (Dowley and O'Sullivan 1985). In B.C., where the population appears to be variable and where both mating types are present, the effect of a reduction in the use of metalaxyl on the prevalence of insensitive strains is unknown.

APPENDIX



Appendix 1. Relative growth of metalaxyl sensitive and insensitive isolates on agar amended with 0, 5, and 30 μ/ml metalaxyl.

Appendix 2. Metalaxyl sensitivity ratio from *in vitro* agar assay compared to *in vivo* floating leaf disc assay for metalaxyl sensitivity (average of 2 trials).

Isolate Identification	Leaf disc area covered with sporangia (%)		Agar 50:0 ratio	
CG96	0	MS	0.0	MS
5.1.7	21	MI*	0.1	MS*
62.1.1	0	MS	0.28	MS
5.1.2	88	MI	0.40	MI
75.1.6	0	MS*	0.41	MI*
19.2.2	57	MI	0.84	MI
34.1.3	71	MI	0.92	MI
96.1.4a	100	MI	0.98	MI
CG215	54	MI	0.99	MI

* Results not in agreement between the two tests.

Appendix 3: Overwintering of *Phytophthora infestans* on tubers in Langley, British Columbia.

Isolate mating type and metalaxyl sensitivity	Number of tubers from which *P. infestans* isolated after: 31 days	73 days	100 days	Mean	
A1 MS	6	1	0	2.3	a
A2 MS	1	2	0	1.7	a
A1 MI	1	1	0	0.7	a
A2 MS	1	0	0	0.3	a

Analysis using bonferonni test with P = 0.05 (SAS, 1988).

Appendix 4. Fitness test data: comparison of sporangia/mm^2 of representative isolates of A1 MS, A1 MI, A2 MS, and A2 MI.

Isolate Identification	**Mating type and metalaxyl sensitivity**	**Mean sporangia/mm^2 of leaf area Trial 1 (n=5)**	**Mean sporangia/mm^2 of leaf area Trial 2 (n=4)**
62.1.1	(A1MS)	86	0
19.2.2	(A1MI)	41	7
75.1.6	(A1MI)	72	110
34.1.3	(A1MI)	18	35
5.1.7	(A2MS)	132	74
5.1.2	(A2MI)	20	48
96.1.4a	(A2MI)	38	67

LITERATURE CITED

Abad, Z. G., Abad, J. A., and Ochoa, C. 1995. Historical evidence that supports the modern theory of the Peruvian Andes as the centre of origin of *Phytophthora infestans*. Pages 239-245 in: *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan, (eds.) Proceedings of the European Association for Potato Research, Ireland.

Andrivon, D., C., Béasse, and C. Laurent, 1994. Characterization of isolates of *Phytophthora infestans* collected in northwestern France from 1988 to 1992. Plant Pathol. 43:471-478.

Bashan, B., Kadish, D., Levy, Y., and, Cohen, Y. 1989. Infectivity to potato, sporangial germination, and respiration of isolates of *Phytophthora infestans* from metalaxyl-sensitive and metalaxyl-resistant populations. Phytopathology 79:832-836.

B.C.M.A.F.F. 1993. 1993-1994 Vegetable production guide for commercial growers. Province of British Columbia Ministry of Agriculture, Fisheries and Food, Extension Systems Branch. Victoria, B.C..

B.C.M.A.F.F. 1995. Vegetable production guide for commercial growers 1995-1996 edition. Province of British Columbia Ministry of Agriculture, Fisheries and Food, Extension Systems Branch. Victoria, B.C.

Bruni, G. C. A., and Edgington, L. V. 1981. Adaptive resistance in *Peronosporales* to metalaxyl. Can. J. Plant Pathol. 3:201-206.

Caten, C. E., and Jinks, J. L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variability. Can. J. Bot. 46:329-348.

Campbell, A.M. and Duncan, J.M. 1985. Production of oospores in vitro by self fertilization in single isolates of A1 mating types of *Phytophthora infestans*. Trans. Br. Mycol. Soc. 84:533-535.

Carlile, M. J. and Watkinson, S. C. 1994. The Fungi. Academic Press Ltd. London.

Cohen, Y., and Coffey, M.D. 1986. Systemic fungicides and the control of *Oomycetes*. Ann. Rev. Phytopathol. 24:311-338.

Cohen, Y., and Samoucha, Y. 1984. Cross resistance to four systemic fungicides in metalaxyl-resistant strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*. Plant Dis. 68:137-139.

Cohen, Y., and Samoucha, Y. 1989. Selection for metalaxyl resistance in potato crops infected with *Phytophthora infestans*: effect of fungicides and initial frequency of resistant sporangia. Plant Pathol. 38:382-390.

Collier, R. A. and LeBoutillier, S. J. 1995. Minimizing phenylamide resistance: a successful strategy in Jersey. Pages 148-153. in *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan, (eds.) Proceedings of the European Association for Potato Research, Ireland.

Courtice, G. R. M. and Ingram, D.S. 1987. Sexual systems in pathogenic fungi. Pages 143-160 in: Genetics and Plant Pathogenesis. P. R. Day and G. J. Jellis, (eds.) Blackwell Scientific Publications, Oxford.

Daggett, S. S., Götz, E., Therrien, C. D. 1993. Phenotypic changes in populations of *Phytophthora infestans* from Eastern Germany. Phytopathology. 83:319-323.

Davidse, L. C. 1982. Acyclalanines: resistance in downy mildews, *Pythium*, and *Phytophthora* spp. Pages 118-127 in: Fungicide resistance in crop protection. J. Dekker and S.G. Georgopoulos, (eds.) Wageningen.

Davidse, L. C., Gerritsma, O. C. M., and Veltuis, G. C. M. 1984. A differential basis of antifungal activity of acylalanine fungicides and structurally related

chloroacetanilide herbicides in *Phytophthora megasperma* f.sp. *medicaginis*. Pesticide Biochem. Physiol. 21:301-308.

Davidse, L.C., Looijen, D., Turkensteen, L.J., and Van Der Wal, D. 1981. Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Neth. J. Plant Pathol. 87: 65-68.

Davidse, L.C., Van Den Berg-Velthuis, G. C. M., Mantel, B. C., and Jespers, A. B. K. 1991. Phenylamides and *Phytophthora*. Pages 349-360 in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D.S. Shaw, and L.R. Cooke, (eds.) Cambridge University Press, Cambridge.

Deahl, K. L., Goth, R. W., Young, R., Sinden, S. L., and Gallegly, M. E. 1991. Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. Am. Potato J. 68:717-725.

Deahl, K. L., Inglis, D. A., and DeMuth, S. P. 1993. Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. Am. Potato J. 70:779-795.

Dekker, J. 1976. Acquired resistance to fungicides. Annual Review of Phytopathology. 14:405-428.

Dekker, J, 1982. Can we estimate the fungicide resistance hazard in the field from laboratory and greenhouse tests. Pages 118-127 in Fungicide resistance in crop protection. J., Dekker, S.G. Georgopoulos, (eds.) Wageningen.

Dowley, L.J. 1988. Factors affecting the survival of metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 30:473-475.

Dowley, L. J. 1987. Factors affecting the survival of metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Res. 30:473-478.

Dowley, L. J., and E. O'Sullivan. 1985. Monitoring metalaxyl resistance in populations of *Phytophthora infestans*. Potato Research. 28:531-534.

Dowley, L. J. and E. O'Sullivan. 1995. Potato late blight control: Technical manual. TEAGASC, Carlow, Ireland.

Drenth, A., Tas, I. C. Q., and Govers, F. 1994a. DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. Eur. J. of Plant Pathol. 100:97-107.

Drenth, A., Tas, I. C. Q., and Govers, F. 1994b. Acquisition of sexual reproduction and rapid evolution of *Phytophthora infestans*. Pages 105-115 in Drenth A. Ph.D. Thesis. Molecular Genetic evidence for a new sexually reproducing population of *Phytophthora infestans* in Europe.

Drenth, A., Janssen, E. M., and Govers, F. 1994c. Formation and survival of oospores of *Phytophthora infestans* under natural conditions. Pages 118-130 in Drenth A. Ph.D. Thesis. Molecular Genetic evidence for a new sexually reproducing population of *Phytophthora infestans* in Europe.

Elliot, G. 1983. Physiology of sexual reproduction. Pages 71-80 in *Phytophthora*, it's biology, taxonomy, ecology, and pathology. D.C. Erin, S. Bartnicki-Garcia, and P.H. Tsao, (eds.) American Phytopathology Society.

Fairclough, R. W., Bain, R. W., and Holmes, S. J. 1995. The prevention of spread of *Phytophthora infestans* during bulk washing of potato stocks. in *Phytophthora infestans* 150. Dowley, L. J., E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan (eds). Proceedings of the European Association for Potato Research. pp.363.

FAO,. 1982. Recommended methods for the detection and measurement of resistance of plant pathogens to fungicides: Method for fungicide resistance in late blight of potato - FAO Method No. 30. in, FAO Plant Protection Bulletin, 30:69-71.

Frinking, H. D., Davidse, L. C., and Limburg, H. 1987. Oospore formation by *Phytophthora infestans* in host tissue after inoculation with isolates of opposite mating type found in the Netherlands. Neth. J. Plant Pathol. 93:147-149.

Fry, W. E. 1982. Principles of plant disease management. Academic Press, New York.

Fry, W. E., A. Drenth, L. Spielman, B. C. Mantel, L. C. Davidse, and S.B. Goodwin. 1991. Population genetic structure of *Phytophthora infestans* in the Netherlands. Phytopathology 81:1330-1336.

Fry, W. E., Goodwin, S. B., Dyer, A. T., Matuszak, J. M., Drenth, A., Tooley, P. W., Sujkowski, L. S., Koh, Y. J., Cohen, B. A., Speilman, L. J., Deahl, K. L., Inglis, D. A., and Sandlan, K. P. 1993. Historical and recent migrations of *Phytophthora infestans*: chronology, pathways, and implications. Plant Dis. 77:653-661.

Fyfe, A. M. and Shaw, D. S. 1992. An analysis of self-fertility in field isolates of *Phytophthora infestans*. Mycol. Res.96:390-394.

Gallegly, M. E. 1968. Genetics of pathogenicity of *Phytophthora infestans*. Annual Review of Phytopathology. 6:375- 396.

Galindo, J. and Gallegly, M. E. 1960. The nature of sexuality in *Phytophthora infestans*. Phytopathology. 50:123-128

Gees, R. and Hohl, H. R.1988. Cytological comparison of specific (R3) and general resistance to late blight in potato leaf tissue. Phytopathology 78:350-357.

Georgopoulos, S. G. 1982. Detection and measurement of fungicide resistance. Pages 24-31 in: Fungicide resistance in crop protection. J. Dekker. and S. G. Georgopoulos, (eds.) Centre for Agricultural Publishing, Wageningen.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E.. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in Northern and Central Mexico. Phytopathology 82:955-961.

Goodwin, S. B., Cohen, B. A., Deahl, K. L., and Fry, W. E. 1994a. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84:553-558.

Goodwin, S. B., Drenth, A., Fry, W. E. 1994b. Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNA's from *Phytophthora infestans*. Pages 47 - 63 in Drenth A., Ph.D. Thesis. Molecular genetic evidence for a new sexually reproducing population of *Phytophthora infestans* in Europe.

Goodwin, S. B., and Fry, W. E. 1994. Continued migration of A2 mating type, metalaxyl-resistant genotypes of *Phytophthora infestans* in the eastern United States and Canada (Abstr.) Phytopathology 84:1371.

Goodwin, S. B., Spielman, L. J., Matuszak, J. M., Bergeron, S. N., and Fry, W. E. 1992. Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in northern and central Mexico. Phytopathology 82:955-961.

Goodwin, S. B., Sujkowski, L. S., and Fry, W. E. 1995. Rapid evolution of pathogenicity of the potato late blight disease fungus. Phytopathology. 85:669-676

Goodwin, S. B., Sujkowski, L. S., Dyer, A. T., Fry, B. A., and Fry, W. E. 1995. Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in Northern North America. Phytopathology 85:473-479.

Grinberger, M., Kadish, D., and Cohen, Y. 1989. Occurrence of the A2 mating type and oospores of *Phytophthora infestans* in potato crops in Israel. Phytoparasitica 17:197-204.

Hermansen, A., and Amundsen, T. 1995. Mating types of *Phytophthora infestans* in Norway. Pages 77-82 in *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan (eds.) Proceedings of the European Association for Potato Research, Ireland.

Hohl, H. R., and Iselin, K. 1984. Strains of *Phytophthora infestans* from Switzerland with A2 mating type behaviour. Trans. Br. Mycol. Soc. 83:529-530.

Holmes, S. J. I., and Channon, A. G. 1984. Studies on metalaxyl-resistant *Phytophthora infestans* in potato crops in south-west Scotland. Plant Pathol. 33:347-354.

Kadish, D. and Y. Cohen. 1988. Estimation of metalaxyl resistance in *Phytophthora infestans*. Phytopathology 78:915-919.

Kadish, D. and Cohen, Y. 1988. Fitness of *Phytophthora infestans* isolates. Phytopathology 78:912-918.

Kadish, D. and Cohen, Y. 1992. Overseasoning of metalaxyl sensitive and metalaxyl resistant isolates of *Phytophthora infestans* in potato tubers. Phytopathology 82: 887 - 889.

Kadish, D., Grinberger, M., and Cohen, Y. 1990. Fitness of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* on susceptible and resistant potato cultivars. Phytopathology 80:200-205.

Koh, Y. J., S. B. Goodwin, S. B. Dyer, A. T. Cohen, B.A. Ogoshi, A., Sato, N. and W. E. Fry. 1994. Migrations and displacements of *Phytophthora infestans* populations in east Asian countries. Phytopathology 84: 922-927.

Ko, W. H. 1994. An alternative possible origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 84:1224-1227.

Lapwood, D. H. 1961. Potato haulm resistance to *Phytophthora infestans*. Ann. Appl. Biol. 49:316-330.

Ligertwood, G. L. and Hinds, H. D. 1995. The potential of improved control of late blight (*Phytophthora infestans*) of potato tubers by improved deposition of fungicides using dropleg application (Abstr.) Page 369 in *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan (eds). Proceedings of the European Association for Potato Research, Ireland.

MacKenzie, D. R., Elliot, V. J., Kidney, B. A., King, E. D., Roger, M. H., and Theberge, R. L. 1983. Applications of modern approaches to the study of the epidemiology of diseases caused by *Phytophthora*. Pages 303-313 in: *Phytophthora*: it's biology, taxonomy, ecology and pathology. D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao, (eds.) American Phytopathology Society, Minnesota.

Malcolmson, J.F. 1985. *Phytophthora infestans* A2 compatibility type recorded in Great Britain. Trans. Br. Mycol. Soc. 85: 531.

Matuszak, J. M., Fernandez-Elquezabal, J., Gu, W. K., Villarreal-Gonzalez, M., and Fry, W. E. 1994. Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: distribution and dynamics. Plant Dis. 78:911-916.

Maufrand, R., Archer, S. A., Buck, K. W.,Shaw, D. S., and Shatttock, R. C.. 1995. The use of RAPD markers in genetic studies of *Phytophthora infestans*. Pages 55-63 in *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan, (eds.) Proceedings of the European Association for Potato Research, Ireland.

Mosa, A. A., Kato, M., Sato, N., Kobayshi, K., and Ogoshi, A. 1989. Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Japan. Ann. Phytopath. Soc. Japan 55:615-620.

National Research Council. 1989. Lost Crops of the Incas: little-known plants of the Andes with promise for worldwide cultivation. National Academy Press, Washington, D.C.

Nelson, E.C. 1995. The cause of the calamity: the discovery of the potato blight in Ireland, 1845-1847, and the role of the National Botanic Gardens, Glasnevin, Dublin. Pages 1-11 in in: *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan, (eds.) Proceedings of the European Association for Potato Research, Ireland.

Niederhauser, J. S. 1989. *Phytophthora infestans*: the Mexican connection. Pages 25-45. in: *Phytophthora*. J. A. Lucas, R. C. Shattock, D. S. Shaw and L. R. Cooke, (eds.) Cambridge University Press, Cambridge.

Ormrod, D., Plant Pathologist, Ministry of Agriculture, Fisheries and Food, Crop Protection Branch, 1767 Angus Campbell Rd., Abbotsford, B.C.

Parry, D. W. Plant pathology in agriculture. Cambridge University Press. Cambridge.

Pittis, J. E., and Shattock, R. C. 1994. Viability, germination and infection potential of oospores of *Phytophthora infestans*. Plant Pathol. 43:387-396.

Platt, H. W. Agriculture Canada, Research Branch, P. O. Box 1210, Charlottetown, Prince Edward Island, Canada.

Platt, H. W.,and McRae, K. B. 1990. Assessment of field responses of potato cultivars and breeder seedlings to potato late blight epidemics. Am. Pot. Jour. 67:427-441.

Platt, H. W., Peters, R. D., Hall, R., Matheson, B., and McNally-Shanahan, L. 1995. Characterization of isolates of *Phytophthora infestans* from Canada (Abstr.) Pages 371-372 in *Phytophthora infestans* 150. L. J.,Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan (eds.) Proceedings of the European Association for Potato Research, Ireland.

Ribeiro, O. K. 1983. Physiology of asexual sporulation and spore germination in P*hytophthora*. Pages 55-70 in: *Phytophthora*, it's biology, taxonomy, ecology, and pathology. D.C. Erwin, S. Bartnicki-Garcia, and P.H. Tsao, (eds.) American Phytopathology Society.

Russell, G. E. 1978. Plant breeding for pest and disease resistance. Butterworths. London.

SAS Institute Inc., 1988. SAS system release 6.03. Cary, N.C.

Shattock, R. C., Shaw, D. S. Fyfe, A. M., Dunn, J. R., Loney, K. H., and Shattock, J. A. 1990. Phenotypes of *Phytophthora infestans* collected in England and Wales from 1985 to 1988: mating type, response to metalaxyl and isozyme analysis. Plant Path. 39:242-248.

Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986a. Genetics of *Phytophthora infestans*: Characterization of single - oospore cultures from A1 isolates induced to self by intraspecific stimulation. Phytopathology. 76:407 - 410.

Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986b. Genetics of *Phytophthora infestans*: Determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology 76: 410 -413.

Shaw, D. S. 1987. The breeding system of *Phytophthora infestans*: the role of the A2 mating type. Pages 161-174 in: Genetics and plant pathogenesis. Day, P. R., and Jellis, G. J. (eds). Blackwell Scientific Publications, Oxford.

Shaw, D. S. 1985. Occurrence of the rare A2 mating type of *Phytophthora infestans* on imported Egyptian potatoes and the production of sexual progeny with A1 mating types from the UK. Plant Path. 34: 552-556.

Shaw, D.S. and Shattock, R.C. 1991. Genetics of *Phytophthora infestans*: the Mendelian approach. Pages 218-230 in *Phytophthora*. Lucas, J. A., R. C. Shattock, D. S. Shaw and L. R. Cooke, (eds.) Cambridge University Press, Cambridge.

Shen, C. Y., Bower, L. A., Erwin, D. C., and Tsao, P.H. 1983. Formation of sex organs in the A1 mating type of *Phytophthora infestans* induced chemically by A2 isolates of other species of *Phytophthora*. Can J. Bot. 61:1462-1466.

Shtienberg, D., Raposo, R., Bergeron, S. N., Legard, D. E., Dyer, A. T., and Fry, W. E. 1994. Incorporation of cultivar resistance in a reduced-sprays strategy to suppress early and late blights on potato. Plant Dis. 78:23-26.

Singh, B. P., Roy, S., and Bhattacharyya, S. K. 1994. Occurrence of the A2 mating type of *Phytophthora infestans*. Pot. Res. 37: 227 - 231.

Sisler, H. D. 1977. Fungicides: Problems and prospects. Pages 531-547 in Antifungal compounds. M. Sigel and H. D. Sisler (eds.) N. Y. Maral Dekker Inc., New York.

Sozzi, D., and Staub, T. 1987. Accuracy of methods to monitor sensitivity of *Phytophthora infestans* to phenylamide fungicides. Plant Dis. 71:422-425.

Speilman, L. J., Drenth, A., Davidse, L. C., Sujkowski, L. J., Gu, W., Tooley, P. W. , and Fry, W. E. 1991. A second world-wide migration and population displacement of *Phytophthora infestans*? Plant Pathol. 40:422-430.

Stevenson, W. R. 1993. Management of early blight and late blight. Pp. 141-147 in Potato Health Management. R. C. Rowe (ed.). American Phytopathology Society, St. Paul, MN.

Staub, T. H., Dahmen, H., and Schwinn, F. J. 1980. Effects of ridomil on the development of *Plasmopara viticola* and *Phytophthora infestans* on their host plants. Journal of Plant Diseases and Protection. 87: 83 - 91.

Sujkowski, L. S., Goodwin, S. B., Dyer, A. T., and Fry, W. E. 1994. Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. Phytopathology 84:201-207.

Therrien, C. D., Ritch, D. L., Sujkowski, L. S., Spielman, L. J., Fry, W. E., Daggett, S. S., Sim, J. H., and Tooley, P. W. 1993. *Phytophthora infestans* in Poland from 1987-1989. Nuclear DNA content, mating type distribution and response to metalaxyl. J. Phytopathol. 139:68-80.

Therrien, C.D., Tooley, P. W., Speilman, L. J., Fry, W. E., Ritch, D. L., and Shelley, S. E. 1993. Nuclear DNA content, allozyme phenotypes and metalaxyl sensitivity of *Phytophthora infestans* from Japan. Mycol. Res. 97: 945-950.

Tooley, P. W. and Carras, M. M. 1995. Development of PCR primers for the detection of *Phytophthora infestans* species infecting potatoes (Abstr.) Page 375 in *Phytophthora infestans* 150. L. J. Dowley, E. Bannon, L. R. Cooke, T. Keane, and E. O'Sullivan, (eds.) Proceedings of the European Association for Potato Research, Ireland.

Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characteriazation of sexual and asexual *Phytophthora infestans* populations. Journal of Heredity. 76:431-435.

Tooley, P. W., Sweigard, J. A. and Fry, W. E. 1986. Fitness and virulence of *Phytophthora infestans* isolates from sexual and asexual populations. Phytopathology 76:1209-1212.

Tooley, P.W., Therrien, C. D., Sim, J. H., O'Sullivan, E., and Dowley, L. J. 1993. Mating type, nuclear DNA content and isozyme genotypes of Irish isolates of *Phytophthora infestans.* Mycol. Res. 97: 1131-1134.

Van der Plank, J. E. 1968. Disease resistance in plants. Academic Press, New York.

Van der Plank, J. E. 1975. Principles of plant infection. Academic Press, New York.

Van Oijen, M. 1992. Evaluation of breeding strategies for resistance and tolerance to late blight in potato by means of simulation. Neth. J. Pl. Path. 98:3-11.

Vartanian, V. G. and Endo, R. M. 1985. Overwintering hosts, compatibility types, and races of *Phytophthora infestans* on tomato in southern California. Plant Dis. 69: 516-519.

Vernon, B. Agriculture Canada, P. O. Box 1000, Pacific Agricultural Research Centre, Aggasiz, British Columbia.

Weier, T. E., Stocking, C. R., Barbour, M. G., Rost, T. L. 1982. Botany 6th edition. John Wiley and Sons, New York.

Zar, J. 1984. Biostatistical Analysis. Prentice-Hall Inc. Englewood Cliffs, New Jersey.