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THESIS

POPULATION BIOLOGY OF *PHYTOPHTHORA INFESTANS* IN NORTHERN THAILAND

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Jiraphan Sopee 2012: Population Biology of *Phytophthora infestans* in Northern Thailand. Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Somsiri Sangchote, Ph.D. 220 pages.

One hundred thirty-two isolates of *Phytophthora infestans* were isolated from infected potato leaflets collecting in the northern Thailand, Chiang Mai and Tak provinces, during 2006-2009 used to study their population biology. These isolates were analyzed for morphology, pathogenicity, sensitivities to metalaxyl fungicide, mating type, mitochondrial DNA haplotype, and variation of three nuclear gene regions (RAS, Intron RAS, and B-Tubulin) and RAPD fingerprinting.

All isolates were studied on their growth and development on modified media using plant ingredients available in Thailand compared with V8 and rye A agar. It showed that black sesame, black bean, and red kidney bean could be as based media for culture and long term storage. Colonial characters of these isolates on rye A agar were cottony (26% of the isolates), powdery (52%), and concentric ring (22%) type. On potato tuber slices, 64% of the isolates were mycelial and 36% sporangial type. The sizes of sporangia and oospores were varied within the population. Pathogenicity test of these isolates on potato leaflets cv. Atlantic and Spunta revealed that 73 and 54% of the isolates produced large lesions, respectively. A selected aggressive Thai isolate could cause disease on the other hosts including petunia, eggplant, and chilli leaves. One hundred twenty isolates were intermediate and 12 isolates were sensitive to metalaxyl. All of these isolates were A1 mating type and mitochondrial haplotype IIa. No variation was observed for DNA gene sequences among isolates. Polymorphism was identified by 17 RAPD primers. A total of 86 bands were amplified by RAPDs, with the similarity coefficient ranging between 0.54 and 0.97.

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POPULATION BIOLOGY OF *PHYTOPHTHORA INFESTANS* IN NORTHERN THAILAND

INTRODUCTION

Late Blight, a disease caused by the destructive pathogen, *Phytophthora infestans*, has been one of the most damaging plant diseases worldwide. Late blight of potato (*Solanum tuberosum* L.) caused by *P. infestans*-infection of leaves, stem and tubers is the most potato disease worldwide. Several additional plant species of the family Solanaceae; tomato, tobacco, egg plant, petunia, tomatillo etc., are also infected by *P. infestans*.

Late blight is a community disease, that is, it is easily spread form farm to farm and over greater distances. Sporangia of *P. infestans* are disseminated by wind from infected leaves to other plants and fields. Epidemics of late blight have become more difficult to manage due to an increasing genetic diversity of this pathogen (Fry and Goodwin, 1997a and 1997b). Late blight management strategies and forecasting systems for this disease depend on an understanding of its genetics because of the relationship of genotypes and epidemiologically important features such as host specificity, cultivar response, fungicide sensitivity, and weather.

The concept of a clonal lineage is important to understanding the population biology. The clonal lineages of *P. infestans* include the asexual and sexual populations. Initial description of a clonal lineage requires a large number of markers. The global markers used to define clonal lineage of *P. infestans* is divided into 2 categories: phenotypic markers (mating type, virulence, fungicide resistance, lesion size, incubation period, sporulation capacity etc.) and genotypic markers (allozyme, restriction fragment length polymorphisms [RFLPs probe RG57], mitochondrial DNA haplotype, amplified fragment length polymorphisms [AFLPs], simple sequence repeat markers [SSRs], single nucleotide polymorphisms [SNPs], sequencing etc.).

The worldwide migration of new biotypes of late blight in potato and tomato crops in recent decades has caused a problem on disease management. Population biology of the causal agent, *P. infestans,* must be first understood before developing a strategy for control and preventing or detecting new biotype. The changes in pathogen populations depend on the meteorological conditions and the differences in potato cultivars in a particular country. Comparing genotypes of *P. infestans* Thai isolates using the global markers have been conducted to fulfill the knowledge of this pathogen in Thailand.

OBJECTIVES

1. To determine the most effective culture media for growth and development of *Phytophthora infestans* in Thailand

2. To discriminate among *Phytophthora infestans* Thai population using morphological and cultural characteristics

3. To study the pathogenicity and the aggressiveness of *Phytophthora infestans* population in Thailand

4. To characterize the population of *Phytophthora infestans* in Thailand using global markers such as mating type, sensitivity to metalaxyl fungicide, and mitochondrial DNA (mtDNA) haplotypes

5. To determine the genetic variation of *Phytophthora infestans* in Thailand using DNA sequencing and RAPD fingerprinting

LITERATURE REVIEW

General Introduction

1. Potato Production in Thailand

The northern and northeastern parts of Thailand is the major area for potato production, approximately 54.40, 25.43 and 8.48% of crop is in Chiang Mai, Tak and Lamphun province, respectively (OAE, 2010). The weather in these areas is suitable for growing potato. The “Atlantic” and “Spunta” varieties are the dominant potatoes grown for chips and fresh consumption (Pongkao and Sinthuprama, n.d).

From the early 1960s through the 1970s, potatoes remained a relatively minor crop in Thailand. The area of cultivation was increased to 1,400 hectares in 1965. Recently, potato cultivation has been used by international agencies as alternative crop opium. The growth of tourist and hotel industry in Bangkok and southern Thailand has further increased the demand of potatoes. In the past five years, 7,000 hectares were being cultivated and produced 90,000 tons annually (Anonymous, 2007a). According to Office of Agricultural Economics (2010), potato production areas in Thailand were 8,133.92 hectares which was 0.01 percent of the world market and yielded 126,386 tons. Although the trend of potato tuber production both for processing and table potato was slightly high during 2007-2009, import of seed potato also increased. Potato production seasons are named according to the season when they are planted and harvested: summer (November to April) and fall (July to October) (Pongkao and Sinthuprama, n.d).

2. Late Blight

Late blight is a major disease of both potatoes and tomatoes. Late blight caused the Irish potato famine in the 1850's. During the period, millions of people in Ireland were starved or forced to emigrate. Entire potato crops rotted in the field or in storage because of late blight infection. Late blight is caused by *P. infestans* that survives from one season to the next in infected potato tubers.

Late Blight Symptoms (Erwin and Ribeiro, 1996; Hooker, 1981; Seaman *et al*., 2006; Stevenson *et al*., 2001)

On potato plants: Late blight lesions can occur on both leaves and stems. The first appearance of lesions commonly occurs after periods of wet weather. Black lesions on the leaves appear within 3-7 days of infection. Under humid conditions, delicate, whitish fungal spore producing structures are produced at the edge of the lesion, particularly on the abaxial side of the leaf (Fig. 1A). Lesions turn brown and surrounded by soft watery of gray-green tissue. Once lesions dry up, the white spore masses will disappear.

On potato tubers: Infected potatoes have shallow, brownish or purplish lesions on the surface of the tuber. Cut across the surface of these infected areas, a reddish-brown, dry, granular rot extends to half an inch into the flesh (Fig. 1B).

On tomatoes: Symptoms on tomato leaves and stems are similar to those on potato. On tomato fruit, late blight causes a firm, dark, greasy looking lesion from which the fungal spore producing structures emerge under humid conditions (Fig. 1C, D).

3. Characteristics of *Phytophthora infestans*

The fungus-like organism, *Phytophthora infestans* (Mont.) de Bary, belongs to the Kingdom Chromista, Phylum Oomycota, Class Perenosporea, Subclass Perenosporidea, Order Perenosporales, Family Perenosporaceae, Genus *Phytophthora* and Species *P. infestans*. Family Perenosporaceae includes aquatic, amphibious, and terrestrial fungi, caused serious diseases of economic plants (Alexopoulos *et al.*, 1996; Anonymous, 2006a; Anonymous, 2006b; Anonymous, 2011).

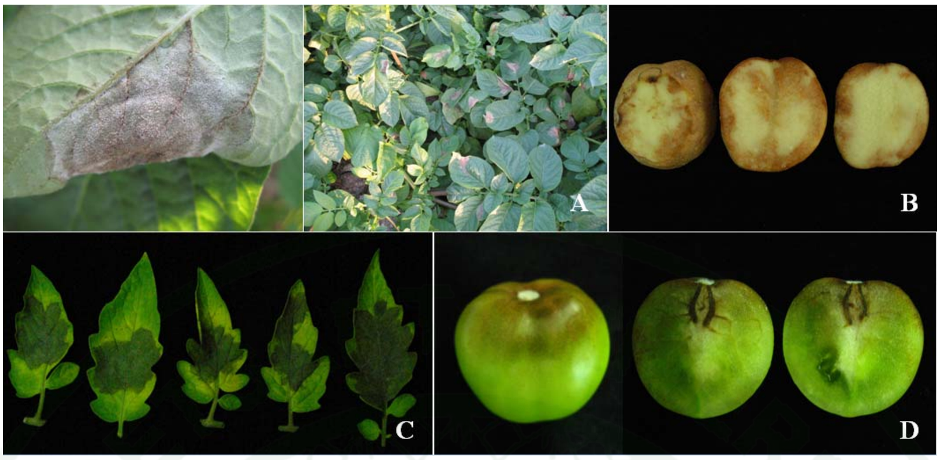

Figure 1 Late blight symptoms on A) potato plant, B) potato tuber, C) tomato plant and D) tomato fruit**.**

P. infestans is classified in group IV Phytophthora species. Sporangia are semipapillate with an apical thickening is shallower than that in group I and II. Sporangia are usually deciduous. New sporangia do not proliferate internally. Sporangiophores are sympodial. Mating types of *P. infestans* are categorized into mating type A1 and A2. Oospores are seldom forming in single isolate culture but form when A1 and A2 mating types are paired. Antheridia are predominantly amphigynous type (Erwin and Ribeiro, 1996).

This fungus-like organism is characterized by coenocytic mycelium and the production of biflagellate, motile zoospores. The sporangia are formed under humid conditions mainly during the night and dispersed under dry conditions during the day by wind. They germinate under humid conditions e.g. in a dew droplet. The zoospores will produce under wet condition and enter the host plant through the stomata and cause new infections (Alexopoulos *et al.*, 1996). *P. infestans* is heterothallic and can reproduce sexually in the presence of the opposite mating type. Sexual reproduction follows fertilization of an oogonium by an antheridium resulting in the production of an oospore. The complicated life cycle (Fig. 2), with very distinct and strikingly different spore forms ranging from motile zoospores to thickwalled oospores, makes control of diseases caused by *Phytophthora* difficult and challenging (Schumann and D'Arcy, 2000; Zentmyer, 1983).

P. infestans is parasitic on members of the Solanaceae, its three major hosts are potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), pear melon (*Solanum muricatum* Ait.) (Erwin and Ribeiro, 1996), and the other host plant species as shown in Table 1.

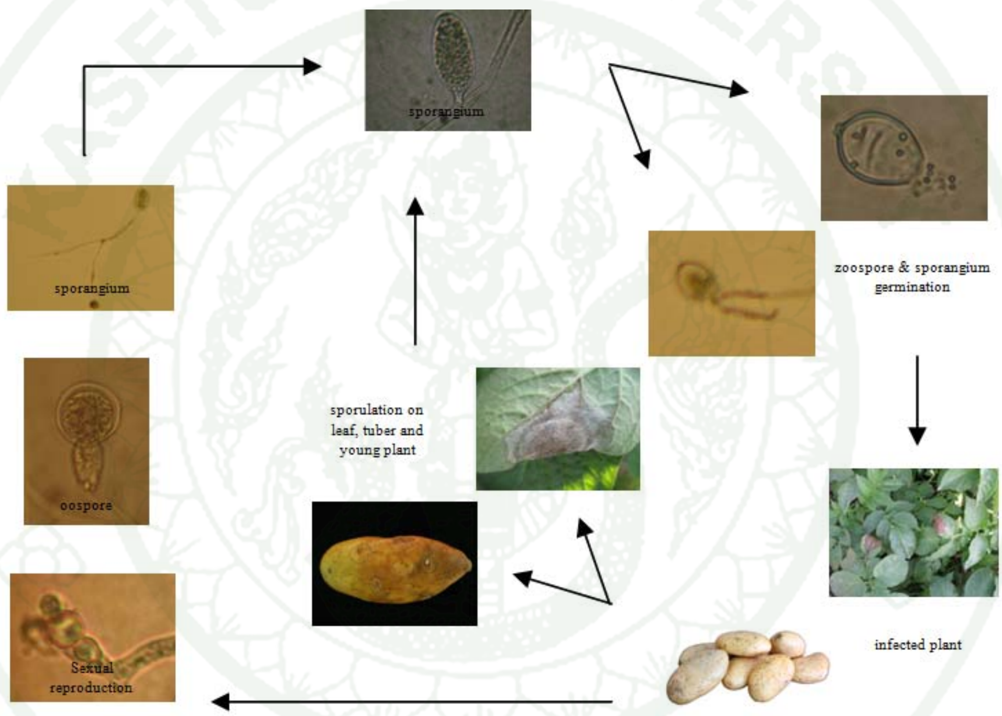

Figure 2 Life cycle of *Phytophthora infestans*, causal agent of late blight of potato and tomato.

P. infestans is disseminated by airborne sporangia (distant spread) and from point sources by water splash of sporangia from infected leaves. Thus, epidemics can result from the dispersal of inoculum from distant fields or from infected seed tubers. Sporangia are produced in higher numbers (millions per plant per season) than oospores (hundreds per plant per season), and can survive outside of living plant tissue; 6-8 months for mycelia, 0.5-2.5 months for sporangia/zoospores and >9 months for oospores (Erwin and Ribeiro, 1996).

4. Historical Migrations of *Phytophthora infestans*

4.1 Worldwide migration

Currently, *P. infestans* distributes into potato and tomato cultivating area throughout the world. Migrations were out of the center of origins on at least four occasions (Fry and Goodwin, 1997a, b; Fry *et al*., 1993; Goodwin, 1997).

The migration from Mexico to the United States during 1840s: *P. infestans* genotypes contain the US-1 clonal lineage (mating type A1, sensitive to metalaxyl, the allozyme alleles *Gpi* 86/100 and *Pep* 92/100, Ib mtDNA haplotype) called old population had distributed from Mexico to the United States composing of 3 steps. First steps in the migration may be involved with oospore-contaminated soil, tuber of wild *Solanum* sp. or infected potato tuber from Mexico was introduced into the United States. Second steps in the migration probably occurred during 1844 or 1845 from the United States into Europe. Third steps in the migration involving on transportation of potato seed from Europe to Africa, Asia, South America and subsequently worldwide.

Table 1 Distribution of hosts of *Phytophthora infestans*

Host	Common name	Disease	Geographical distribution
Capsicum annum	Red pepper	Leaf blight	United States (Cox, 1948)
Lycopersicon esculentum	Tomato	Late blight, Damping-off	World wild distribution by 1930 (Tucker, 1933)
Nicotiana sp.	Tobacco	Leaf blight	United States (Vartanian & Endo, 1985)
Solanum melongena	Eggplant	Fruit rot and calyx blight	United States (Haskell, 1921)
Solanum tuberosum	Potato	Late blight	World wild distribution by 1930 (Tucker, 1933)
Schizanthus sp.	Butterfly flower	Stem, leaf and bud blight	United States (Reddick, 1928)
Ipomoea hederacea	Morning-glory	Leaf blight	India (Raj *et al*., 1976)

The migration of *P. infestans* out of Mexico appears to have occurred during the late 1970s: New clonal lineage US-6 (mating type A1, sensitive and resistant to metalaxyl, the allozyme alleles *Gpi* 100/100 and *Pep* 92/100, IIb mtDNA haplotype) migrated from northwestern Mexico into the United States.

The migration of *P. infestans* out of Mexico appears to have occurred during the late 1980s: Mating type A2 was commonly found in Mexico while mating type A1 was common in worldwide with rare or absent of A2. During 1976 and 1977, a total 25,000 tons of potatoes were shipped from Mexico to Europe. Most studies revealed that the mating type A2 with new allozyme genotypes had occurred in the Netherlands, eastern Germany, throughout Europe, Middle East, Africa and South America.

The migration of *P. infestans* out of Mexico into the United States and Canada: Two new genotypes, US-7 (mating type A2, highly resistant to metalaxyl, the allozyme alleles *Gpi* 100/111 and *Pep* 100/100, Ia mtDNA haplotype) and US-8, (mating type A2, highly resistant to metalaxyl, the allozyme alleles *Gpi* 100/111/122 and *Pep* 100/100, Ia mtDNA haplotype) were detected in the United States beginning in 1992 and Canada by 1994.

4.2 Center of Origins

Mexico has been proposed as the center of origin and diversity of the late blight pathogen. The A1 mating type had been distributed to elsewhere in the world before 1980, while both A1 and A2 mating types were reported only in Mexico (Fry and Goodwin, 1997; Goodwin *et al*., 1994). Reproduction in *P. infestans* is primarily asexual, sexual reproduction being mainly restricted to Mexico. *P. infestans* is heterothallic and requires two mating types, A1 and A2 for sexual reproduction. Prior to the 1980's the A2 mating type was restricted to Mexico. This region is considered the center of origin of this fungus and both mating types occurred in approximately equal frequency. The first reports of the A2 mating type outside Mexico were from

Switzerland in 1984 and from U.K. in 1985. Since then, it has been reported from the Netherlands, Israel, Japan, Egypt, and India (Singh *et al.* 1993).

There are 3 theory proposes for the center of origin of the late blight pathogen. One theory proposes that the center of origin and diversity of the late blight pathogen is Mexico which provided the source of inoculum for the late blight epidemics of the 1840s. A second theory proposes that the center of origin and the source of inoculum for 19^{th} century late blight epidemics are originated from Peru. A third theory proposes that the Mexico represents the center of origin of the late blight pathogens, but Peru is the source of inoculums for 19^{th} century epidemics in Europe and United State (Ristaino, 2002).

Chapter 1
Culture Media for *Phytophthora infestans*

The oomycete pathogen *Phytophthora infestans* (Mont.) de Bary causes an economically destructive disease of potato (*Solanum tuberosum* L.), potato late blight, (Stevenson *et al*., 2001) and has worldwide distribution throughout areas of intense potato production (Shattock *et al*. 1990; Shrestha *et al*., 1998; Nishimura *et al*., 1999; Sedegui *et al*., 2000; Derie and Inglis, 2001; McLeod *et al*., 2001; Reis *et al*., 2003). In 2009, potatoes were produced in Thailand on about 8,134 hectares which was 0.01 percent of the world market (OAE, 2010). Although, potato production occurs in small relatively isolated areas, the crop is commonly infected by *P. infestans* (Gotoh *et al*., 2005) which causes significant damage. Dowley *et al*. examined yield loss from late blight in Ireland from 1983 to 2007 and found an average loss of 10.1 t/ha. Changes in the *P. infestans* populations in the early 1980s resulted in the occurrence of new highly pathogenic strains (Gotoh *et al*., 2005). There have been numerous pathological and physiological studies of this pathogen in Thailand (Sanyong *et al*., 1993; Nishimura *et al*. 1999; Gotoh *et al*., 2005; Petchaboon *et al*., 2006, Prakob *et al*., 2007; Jaimasit and Prakob, 2010).

P. infestans is difficult to culture on general media (Dickinson and Keay, 1948). Several semi-synthetic and/or organic media have been developed to support fungal growth, sporulation and long-term storage (Erwin and Ribeiro, 1996). Many substrates have been used for agar-based media that include rye seeds (Caten and Jinks, 1968), sweet corn (Goth, 1981), pea seeds (Hollomon, 1966; Sanyong *et al*., 1993; Hartman and Huang, 1995; Peters *et al*., 1998), soybean and carrot (Medina and Platt, 1999), field corn (Peters *et al*., 1998), bean meal (Sakai, 1959), chick pea (Sanyong *et al*., 1993), oat meal (Savage *et al*., 1968; Skidmore *et al*., 1984), cereal grains and V8 juice (Snieszko *et al*., 1947), and lima bean (Thurston, 1957).

Various media have been compared with rye agar to determine their effect on mycelial growth, sporangial and oospore production, and long-term survival of *P. infestans*. Oat meal agar amended with or without *β*-sitosterol and clarified V8 juice agar with *β*-sitosterol has been used as excellent media for mycelial growth. Sporagial and oospore production were significantly induced on clarified V8 juice and carrot agar containing *β*-sitosterol compared with rye A agar (Medina and Platt, 1999). Sweet corn-based medium has been used to maintain *P. infestans* up to 12 months (Goth, 1981). Corn and rye-based media have shown *P. infestans* survival of more than 30 months (Peters *et al*., 1998). Ground dry bean (*Phaseolus vulgaris* L. “Kintoki”) meal agar supported production of high numbers of sporangia (Sakai, 1959). Alanine added to potato dextrose agar containing 1 percent sucrose showed a slight benefit on mycelial growth, but greatly improved sporulation (Sakai, 1959).

Rye seed has been used for preparing *P. infestans* culture media (Caten and Jinks, 1968; Goth, 1981; Peters *et al*., 1998) and is the most common organic substrate-based medium (Snieszko *et al*., 1947; Hartman and Huang, 1995). An important component, rye grain is not commonly available in Thailand and is costly to import. V8 juice is also widely used as a culture medium for *Phytophthora* spp., but some isolates of *P. infestans* do not grow and sporulate very well on this medium (Snieszko *et al*., 1947). Oat meal agar is a worthy medium for all *Phytophthora* spp., but it’s rather thick and opaque (Savage *et al*., 1968). The most commonly available medium, potato dextrose agar, does not support sporangial production and oospore formation of *Phytophthora* spp. (Hendrix, 1964; Erwin and Ribeiro, 1996).

In Thailand, the main substrates used for preparing culture media for *P. infestans* are not available. Those ingredients that are locally available for the culture of *P. infestans* were evaluated in a preliminary screen (Sopee, 2008). The purpose of the current study was to further evaluate various media using substrates commonly available in Thailand for growth, oospore and sporangia production and long-term storage of *P. infestans*.

Chapter 2
Morphological Characteristics of *Phytophthora infestans*-Thai Isolates

The genus of *Phytophthora* is divided into six groups providing a practical classification (Waterhouse, 1963). *P. infestans* belongs to group IV producing asexual spore called sporangium and sexual spore called oospore which forms after paring of A1 and A2 mating types (Erwin and Ribeiro, 1996). *P. infestans* exchanges genetic material via mutation (Bosmans, 2009), sexual (Anderson, 2007) and parasexual recombinations (Cherepennikova-Anikina *et al*., 2002).

The genetic diversity in *P. infestans* species from the 1840s to the 1970s were dominated by a single lineage, A1 mating type, and performed on a tiny range. After the 1980s, A2 mating type escaped from the center of origin, Mexico, to worldwide changing in the genetics of *P. infestans* population (Fry and Goodwin, 1997a and 1997b). The possibility that immigrant strains might be more aggressive (Davidse *et al*., 1981). Subsequently, Deahl *et al*. (1991, 1993) reported A2 mating type isolates with metalaxyl resistance in the United State and Canada. Shaw *et al*. (2007) reported that a new strain of A2 mating type had fully resistant to metalaxyl. This shows sexual recombination will results in an increasing of genetic variation. Asexual reproduction will maintain and multiply successful genotypes. However, asexual reproduction of *P. infestans* was able to form different clonal lineages that could infect potato cultivars with race specific resistance and develop fungicide resistance

(McDonald and Linde, 2002). The exchange of genetic material through three mechanisms might be involved in phenotypic character of *P. infestans* including sensitivity to fungicide, aggressiveness, capable of sporulation, latent period, morphological change etc.

Despite the worldwide impact of *P. infestans*, understanding of phenotypic variation among isolates in population need further investigation. Since then, there have been numerous reports of *Phytophthora* spp. on macro-morphology: colony type and growth rate, etc., and micro-morphology: sporangium, oospore, gametangium, chlamydospore, etc. Some of the study with *Phytophthora* spp. indicated the existence of host specialization (López-Herrera and Pérez-Jiménez, 1995; Hantula *et al*., 2000), resistant to fungicide or antibiotic (Shaw and Elliott, 1968; Chang and Ko, 1992; Zheng and Ko, 1997), phenotypic differences among the oospores (Kaosiri *et al*., 1980; Chee, 1973), or growth rate at various temperature (Huberli, 2001), temperature requirement species (Bower *et al*., 2007).

Micro-morphologies have been used as a differentiated parameter for *P. infestans*. Three different populations of *P. infestans* (US-1, US-7 and US-8) had shown to vary in sporangial germination (Mitzubuti and Fry, 1998). Kato *et al.* (1992) investigated on culture characteristics of 300 isolates of *P. infestans* on potato tuber slices which were divided into 2 groups, S and M types. The S type grew well on V8 juice agar and coincided with mating type A1. Flier *et al.* (2001) determined variation of oospore formation between intra- and inter-population of *P. infestans* obtained from three-different infected *Solanum demissum* and found that isolates differed in their capability to form oospores. Most crosses produced large numbers of oospores. The sizes of sporangium and oospore were variance in among *P. infestans* Thai-population (Mekmok, 2009; Pinitkul, 2010). Petchaboon (2003) showed difference on size of oospore collected from tomato and potato which cultivated in Tak and ChiangMai provinces. Sopee *et al.* (2011a) revealed on variation on size of pedicel, sporangium and oospore of *P. infestans* isolates and analyzed on relationship of size of these propagules with metalaxyl sensitivity. The result showing this population had variant size of propagules with no relationship between size and metalaxyl sensitivity.

Sopee *et al*. (2011b) compared growth rate of 2 populations, parental isolate and hyphal tip isolate which obtained from cutting hyphal tip of parental isolate, and cultured on metalaxyl amended agar. Twenty three percent of hyphal tip isolates responded differently from parental isolates.

The purpose of the current study was to discriminate among Thai population of *P. infestans* using morphological and cultural characteristics.

Chapter 3
Aggressiveness and Pathogenicity of *Phytophthora infestans*

Phytophthora infestans is an economically important pathogen of potato and tomato which are members of the Solanaceous plants. List of other plants have been reviewed by Erwin and Ribeiro (1996) and Stevenson *et al*. (2001). *P. infestans* can infect all parts of the potato plant: foliage, stem and tuber, as well as tomato.

In 1981 on September, the first epidemic of late blight in Thailand had been reported on local variety of tomato which was cultivated in the experimental field of PaKia Highland Research and Development Center, Chiang Dao district, Chiang Mai province. Tomato plants cv. VF134 were subsequently planted near the infected tomato field and destroyed by late blight disease. In November, late blight infected tomato (cv. VF134) fields were found in Kangsadarn farm, Mae Rim district, Chiang Mai province, the experimental field of Maejo University, San Sai district, Chiang Mai province and tomato field in San Sai district. Later, late blight was also destroyed potato plants cv. Bintje and Mafrona which cultivated in Fang Horticultural Research Unit, Office of Agricultural Research and Development Region 1, Fang district, Chiang Mai province (Sardsud *et al.*, 1982).

Since mid to late 1980s, a new population of *P. infestans* both A1 and A2 strains were continuously appeared in many parts of world such as Europe (Spielman *et al*., 1991), United State (Fry *et al.*, 1993) and Asia (Nishimura *et al*., 1999). The new population contains a different genetic trait from old population. The new strain is more aggressiveness than the old strain (Fry and Goodwin, 1997a; Fry *et al*., 1993; Inglis *et al*., 1996; Miller *et al*., 1998; Spielman *et al*., 1991). Cultural and chemical means using for disease prevention or protection have limited in effectiveness due to the new strain has a characteristic of phenylamide resistance (Fry *et al*., 1992; Gisi and Cohen, 1996). Numerous studies have investigated on relationship between aggressiveness, fungicide sensitivity, host resistance and mating type (Dowley, 1987; Dowley *et al*., 2002; Flier *et al*., 1998; Gisi and Cohen, 1996; Grinberger *et al*., 1995)

1. Aggressiveness on Potato Foliage and Tuber

The aggressiveness of a pathogen is a quantitative measure of its ability to attack a host. The aggressiveness of the *Phytophthora infestans* has a specific trait related to the amount of disease on a particular host which depends on its resistance. Several parameters are generally scored; such as the percentage of effective spores (infection frequency), the time between infection and spore production (latent period), the numbers and duration of spore release (sporulation capacity) (Andrivon, 1993), lesion area (Pliakhnevich and Ivaniuk, 2008), and lesion expansion rate, and incubation period (Suassuna *et al*., 2004). The aggressiveness parameters of an isolate are compared to another. Since aggressiveness depends on the response of potato cultivar foliage and pathogen so it's not related to tuber. It is necessary to measure aggressiveness to foliage and to tubers separately (Andrivon, 1993).

Lebecka *et al*. (2007) inoculated 165 Polish isolates of *P. infestans* on potato leaflets cv. Tarpan. Mean rAUDPC on tested leaflets ranged from 0.242 to 0.338. Mean infection frequency was 99.6%. Pliakhnevich and Ivaniuk (2008) collected 50 *P. infestans* isolates in all Belarus regions to evaluate aggressiveness on potato tuber slices cv. Delphin and these isolates varied greatly but differed insignificantly between the regions of collection. Mukalazi *et al*. (2001) found variation on aggressiveness of 61 isolates collected from different areas of Uganda shown on potato leaflets cv. Victoria.

Flier *et al.* (2001) inoculated 4 aggressive isolates of *P. infestans* on whole potato tuber cv. Astarte, Bintje, Elkana, Elles, Florijn, Kartel, Producent and Seresta. They found that isolate F95573 had the highest invasive ability and caused highest tuber rots. Different potato cultivar variably responded to aggressive isolates. Kirk *et al*. (2001) reported that US-8 isolate produced larger lesion than US-1 isolate after inoculating in apical end of potato tuber cv. Russet Burbank, Superior and Snowden. The variation for foliar aggressiveness exits within the population of *P. infestans* has been reported such as Carlisle *et al*. (2002), Corbiere *et al*. (2010), Oyarzun *et al*. (1998) and Sujkowski (1986). An aggressiveness was also used to differentiate *P. infestans* population have been reported by Flier *et al.* (2007), Jaime-Garcia *et al.* (2000) and Lebreton *et al*. (1999).

A part from isolates of *P. infestans*, aggressiveness also related to progeny and metalaxyl resistance. Fontem *et al.* (2005) revealed that *P. infestans* population obtained in 2002 had higher severity of late blight on potato leaflets cv. Hydra than population in 2001. Anonymous (2003) reported that some progeny of the UK x UK mating were more aggressive than parental isolates. In contrast, Mayton *et al*. (2000) had inoculated zoospore of progeny and parental isolates on to leaflets of potato cv. Superior and tomato cv. Sunrise. The most lesions produced by progeny were smaller and developed more slowly than parental isolate.

Kadish and Cohen (1988) reported that metalaxyl-resistant isolates produced larger lesions in potato leaflets cv. Alpha than metalaxyl-sensitive isolates. The increasing aggressiveness of the isolates corresponding with their resistance to metalaxyl had been investigated as well as Fontem *et al.* (2005).

2. Pathogenicity

Pathogenicity relates to the ability of one organism (the pathogen) to cause disease on another (the host). Clearly, *P. infestans* is able to infect and cause damage to potato and a range of other solanaceous plants but not cereals or oilseed rape (Cooke *et al*., 2006).

Fontem *et al.* (2005) collected 233 isolates from huckleberry, potato and tomato. The pathogenicity tests showed that the potato, tomato and huckleberry isolates infected their primary hosts as well as the others two hosts. Chen *et al*. (2009) inoculated 655 isolates of *P. infestans* obtained from tomato and potato in Taiwan onto tomato seedlings (line CL5915) and potato plants (cv. Kennebec). Isolates collected from tomato before 1997 were aggressive to tomato but not potato. Most isolates obtained after 1998 were aggressive to both hosts. Ann *et al*. (1998) isolated *P. infestans* from tomato and potato and then crossing inoculated to both host. Tomato isolates caused only leaf blight of tomato whereas potato isolates were able to cause the disease both tomato and potato. However, all 177 *P. infestans* isolates collecting in Taiwan during 2004 and 2005 were aggressive on both potato and tomato (Chen *et al*., 2008). Kenneth *et al*. (2006) collected *P. infestans* isolates from 4 solanaceous hosts; black nightshade, hairy nightshade, petunia and tomato, in US and UK. They found that both weeds and cultivated solanaceous species can be infected with *P. infestans* and may serve as source of inoculums. Some of these plants do not show conspicuous symptoms.

Mayton *et al*. (2000) used progeny, oospore, producing from a cross between US-17 and US-8 genotypes, to inoculate leaflets of potato cv. Superior and tomato cv. Sunrise. Oospores were able to infect both tomato and potato leaflets but most lesions were smaller and developed more slowly than those produced by parental isolate. Mukalazi *et al.* (2001) collected 61 isolates of *P. infestans* from late blight infected potato, and then, inoculated by cross infection between potato and tomato hosts. Twenty seven of the isolates infected on tomato. Erselius *et al.* (1998) collected *P. infestans* potato- and tomato-isolates in Uganda and Kenya and then, inoculated onto leaflets of potato cv. Yungay, Cruza-148 and Chata and tomato cv. Caribe, Flora Dade and FMX-193. They found that the host specificity is quantitative rather than qualitative because isolates were more aggressive on their primary host.

Oyarzun *et al.* (1998) inoculated 17 aggressive *P. infestans* isolates which obtained from tomato and potato onto leaflets of potato cv. Uvilla, Bolona and Yema de Huevo and tomato cv. Flora Dade, FMX-93 and Pieralbo. All isolates produced larger lesion on the host from which they were isolated. No isolates were highly aggressive on both tomato and potato. Lebreton *et al*. (1999) collected *P. infestans* in France from potato and tomato and inoculated onto leaflets of potato cv. Bintje and tomato cv. Marmande. They found its pathogenicity on potato and tomato is different between *P. infestans* populations obtain from potato and tomato.

Chapter 4
Genetic Distribution and Characterization of *Phytophthora infestans*

The migration of *P. infestans* from Mexico which is the center of origin of *P. infestans* because both mating types (A1 and A2) are present and there is a high genetic diversity for this pathogen in this region. Sexual reproductions of the pathogen have contributed to genetic changes of the pathogen.

Disease increases have been attributed to an increasing of resistance to fungicide metalaxyl and associated with genetic changes in populations of *P. infestans* (Wangsomboondee *et al*., 2002). In the current, differences of phenotypic and genotypic characteristics among population have been used to discriminate and characterize between new and old population of *P. infestans* (Cooke and Lees, 2004; Forbes *et al*., 1998). The methods such as mating type (A1 and A2), sensitivity to metalaxyl, allozyme genotype at the *glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) loci, and DNA fingerprinting with the RG57 probe, RFLP (Restriction fragment length polymorphism) fingerprinting, RFLP-PCR (Restriction fragment length polymorphism-polymerase chain reaction), AFLP (Amplified [restriction] fragment length polymorphism) and SSR (Simple sequence repeats, microsatellites) etc.

1. Mating Type

Both A1 and A2 mating types of *P. infestans* occur in Mexico, the center of diversity of the fungus (Fry *et al*., 1993; Goodwin *et al*., 1994). The A1 mating type has been found worldwide in all populations of the late blight pathogen outside of

Mexico prior to 1984 (Fry *et al*., 1993). Changing in population of *P. infestans* has been detected in 1984 when isolates with the A2 mating type has been found in Europe and subsequently worldwide as shown in Table 2 (Anonymous, 2007b; Drenth *et al*., 1993; Koh *et al*., 1994). Genetic variation is further increased by an appearance of the A2 mating type which permits sexual hybridization and genetic recombination. The development of a fitter and more aggressive strain of *P. infestans* after sexual reproduction is expected that might be more difficult to control with current fungicides (Dowley *et al*., 2000).

To test mating type, an unknown isolate will be paired with a known tester A1 and A2 isolates and then observed for oospore production. The A1, A2 and self-fertile mating types among the 26 isolates collecting from Ireland between 1988 and 1989 were 62, 35 and 3% of frequency, respectively (Dowley, 2000). All 204 isolates from Northern Ireland between 1998 and 2002 were A1 isolates (Cooke *et al*., 2006). The 1,048 isolates collecting from Netherlands between 1993 and 1996 were 84 and 16% of A1 and A2 isolates, respectively. The overall percentage of A2 isolates in 1996 (56%) was 14 times higher than in 1994 (4%) (Zwankhuizen *et al*., 2000). The 2005 population of *P. infestans* A2 isolates in Great Britain had increased in frequency from 5% to 38% of sites sampled in the last three years (Shaw *et al*., 2007). Mating types of *P. infestans* population had been characterized in many countries in Europe (Flier *et al*., 2007; Lebecka *et al*., 2007; Lehtinen *et al*., 2007; Runno-Paurson *et al*., 2009; Tosun *et al*., 2007), Canada, South and North America (Abu-El Samen *et al*., 2003; Alder *et al*., 2004; Chycoski and Punja, 1996; Fraser *et al.*, 1999; Goodwin *et al*., 1994; Goodwin *et al*., 1998; Goodwin *et al*., 1995; Jaime-Garcia *et al*., 2000; Marshall-Farrar *et al*., 1998; Peters *et al*., 2001; Reis *et al*., 2003; Wangsomboondee *et al*., 2002; Widmark *et al*., 2007), Africa (McLeod *et al*., 2001; Sedegui *et al*., nd), and Asia (Ann *et al*., 1998; Ghimire *et al*. 2001; Gotoh *et al*., 2005; Hartman and Huang, 1995; Jaimasit and Prakob, 2010; Koh *et al*., 1994; Sanyong *et al*., 1993).

2. Virulence

A physiological race of *P. infestans* isolate can be determined by inoculating an isolate onto a series of 11 different potato genotypes which carry a specific R gene, and then scoring for the compatible or incompatible reaction (Cooke and Lees, 2004).

The common pathotypes were 1.3.4.7.8.10.11 and 1.3.4.7.10.11, representing 12% of 101 Estonian isolates during 2002-2003 (Runno-Paurson *et al*., 2009). Twenty different pathotypes were discovered in 196 Finnish isolates during 1997-2000. The 1,3,4,7,10,11 pathotype was common representing 49% of these isolates (Lehtinen *et al*., 2007). The 125 Polish isolates collected in 2005 and 2006 were virulent to genes *R*1, *R*3, *R*4, *R*7, *R*10 and *R*11 at high frequency (above 88%) (Lebecka *et al*., 2007). The virulence of *P. infestans* population had been studied in many countries in Europe (Davidse *et al*., 1983; Dowley *et al*., 2000; Flier *et al*., 2007), United States (Abu-El Samen *et al*., 2003), and Asia (Hartman and Huang, 1995; Sanyong *et al*., 1993; Zhang and Kim, 2007).

3. Phenylamide Resistance

Metalaxyl fungicides had been used to control the late blight since 1977 (Urech *et al*., 1977). The next few years, the metalaxyl resistant of *P. infestans* isolates was first reported in Ireland (Dowley and O'Sullivan, 1981) which widly used metalaxyl to control this disease. The mode of action of phenylamide fungicides such as metalaxyl, oxadixyl, benalaxyl, mefenoxam and ofurace inhibit of RNA synthesis (Gisi and Cohen, 1996). Various methods for assessing resistance had been reviewed by Goth and Keane (1997), Kadish and Cohen (1988), Kadish *et al*. (1990), Sedegui *et al*. (1999) and Sozzi and Staub (1987) by using such as potato leaf disc, detached leaf, whole plant or poisoned food techniques.

Table 2 Distribution of *Phytophthora infestans* A1 and A2 mating types

Region	Country	Population
Africa	Ethiopia,	A1, new population
	Kenya, Uganda	A1, clonal, host specific, old population
	Egypt, Morocco	A1+ A2
	South Africa	A1, clonal, old population
Asia and the Pacific	China, India, Japan, Korea, Nepal, Pakistan, Thailand	A1 + A2
	Bangladesh, Sri Lanka, Philippines, Taiwan, Vietnam	A1
	Indonesia	A2
	Russia	clonal (Siberia) recombinant (near Moscow)
Latin America	Argentina, Brazil, araguay,Uruguay	A1+A2, clonal, host specific
	Bolivia	A2, clonal, host specific
	Chile, Ecuador, Peru,	A1 clonal, host specific
	Colombia, Costa Rica, Honduras, Panama, Venezuela	A1, clonal
	Mexico	A1 + A2 recombinant
Australia	Australia	A1
Europe and East Europe	Austria, Belgium, Denmark, France, Germany, Ireland, Italy, Switzerland, UK-England & Wales	A1 + A2
	Finland, Hungary, Norway, Netherlands, Poland, Sweden, UK-Scotland	A1 + A2 recombinant
	Spain	A1
	UK-Northern Ireland	A1 [A2 not detected since 1996]
USA and Canada	USA	A1 & A2 recombinant
	Canada	A1 & A2 recombinant

Source: Anonymous (2007b), Drenth *et al*. (1993), Koh *et al*. (1994)

Two hundred thirteen isolates of *P. infestans* in North Carolina between 1993 and 1998 expressed range of sensitivity to metalaxyl by showing 12.2% sensitive, 26.8% intermediate and 61% resistant (Wangsomboondee *et al*., 2002). Of 122 isolates tested collecting in Southern and Southeastern Brazil from 1998 to 2000 were ranked as sensitive (35.2%), intermediate (48.4%) and resistant (16.4%) to metalaxyl (Reis *et al*., 2003). Furthermore, the isolates carried metalaxyl-resistant characters had been reported from many countries in Europe (Cooke *et al*., 2006; Dowley *et al*., 2000; Lebecka *et al*., 2007; Lehtinen *et al*., 2007; Runno-Paurson *et al*., 2009), Canada, South and North America (Abu-El Samen *et al*., 2003; Alder *et al*., 2004; Chycoski and Punja, 1996; Dorrance *et al*., 1999; Fraser *et al*., 1999; Goodwin *et al*., 1998; Jaime-Garcia *et al*., 2000; Marshall-Farrar *et al*., 1998; Peters *et al*., 2001; Tombolato, 2002), Africa (McLeod *et al*., 2001; Sedegui *et al*., nd), and Asia (Ghimire *et al*., 2001; Hartman and Huang, 1995; Jaimasit and Pakob, 2010; Koh *et al*., 1994).

4. Allozyme Genotype

The genetic structure of populations of this fungus-like organism can be determined by selectively neutral genetic markers. Allozyme analysis has become an effective tool for identifying variation in *P. infestans* populations. Allozymes are relatively inexpensive markers and easy to use. *Glucose-6-phosphate isomerase* (*Gpi*) and *peptidase* (*Pep*) have been widely used for *P. infestans* population genetics studies because these enzymes provide sufficient polymorphism and clear resolution (Cooke and Lees, 2004; Forbes *et al*., 1998).

Gpi is a glycolic enzyme that catalyzes the reversible isomerization of glucose-6-phosphate to fructose-6-phosphate. Pepidase compise two groups of enzymes that cleave peptide bonds at points within the protein, the endopepidase, and remove amino acids sequentially from either the N- or C-terminus, the exopeptidases (Tombolato, 2002).

The population of *P. infestans* quickly changed between 1992 and 1995 in Oregon and Washington from a population comprised almost exclusively of the US-1 genotype to a population represented by new or recombinant genotypes. A1 isolates with *Gpi 86*/*100* were found in 1993. In 1995, A2 type with *Gpi 100*/*122*, A2 type, metalaxyl resistant isolate with *Gpi 100*/*100*/*111* and A1 isolates with *Gpi 100*/*111*/*122* were found (Miller *et al*., 1997). In 1998-2002, all *P. infestans* isolates in the Northern Ireland were A1 mating type and *Gpi100/100*. The majority was *Pep 100/100* but four *Pep 83/100* and six *Pep 96/100* isolates were identified (Cook *et al*., 2006). Allozyme assay of *P. infestans* population had been characterized in many countries in Europe (Cooke *et al*., 2006; Runno-Paurson *et al*., 2009), Canada, South and North America (Abu-El Samen *et al*., 2003; Fraser *et al.*, 1999; Goodwin *et al*., 1994; Goodwin *et al*., 1998; Goodwin *et al*., 1995; Jaime-Garcia *et al*., 2000; Marshall-Farrar *et al*., 1998; Peters *et al*., 2001; Reis *et al*., 2003; Tombolato, 2002; Wangsomboondee *et al*., 2002), Africa (McLeod *et al*., 2001; Sedegui *et al*., nd), and Asia (Gotoh *et al*., 2005; Koh *et al*., 1994).

5. RFLP-RG57 (Restriction Fragment Length Polymorphism) Fingerprinting

The RFLP-RG57 method, developed in the 1990's, significantly accelerated population studies of the late blight agent and its clonal lineages**.** Nuclear DNA fingerprint with the highly polymorphic, moderately repetitive probe RG57 was completed for 26 selected isolates based on origin, host and preliminary AFLP. Probe RG57 reveals more than 25 different bands in *P. infestans*, most of which represent alleles at independent genetic loci (Goodwin *et al*., 1992).

RFLP analysis with the probe RG57 gives further discrimination of genotypes within an allozyme genotype. In the 1998 to 2002, analysis of 91 isolates *P. infestans* in the Northern Ireland revealed nine RG57 genotypes, three associated with haplotype IIa and six with haplotype Ia. The most common RG57 genotype (51% of isolates) comprised both metalaxyl-resistant and -sensitive haplotype IIa isolates (Cooke *et al*, 2006). RFLP-RG57 of *P. infestans* population had been studied in many countries in Europe (Cooke *et al*., 2006; Dowley *et al*., 2000; Ristaino *et al*., 2001; Runno-Paurson *et al*., 2009; Shaw *et al*., 2007; Zwankhuizen *et al*., 2000), Canada, South and North America (Abu-El Samen *et al*., 2003; Alder *et al*., 2004; Dorrance *et al*., 1999; Goodwin *et al*., 1994; Goodwin *et al*., 1995; Jaime-Garcia *et al*., 2000; Reis *et al*., 2003; Tombolato, 2002; Wangsomboondee *et al*., 2002), Africa (McLeod *et al*., 2001), and Asia (Ghimire et al., 2003; Gotoh *et al*., 2005; Koh *et al*., 1994).

6. RFLP-PCR

Most hypotheses concerning the population genetics of *P. infestans* emerged from analyses of nuclear DNA (nDNA), but recently available data on mitochondrial DNA (mtDNA) generate new findings. During the last decade, much has been learned about mtDNA of oomycetes in general and *P. infestans* in particular. In addition, stramenopile mtDNAs have low variability in genome size and gene complement. A report of limited mitochondrial diversity in *P. infestans* has been confirmed several times via two systems of nomenclature. Carter *et al.* (1990) used RFLP analysis to describe four mtDNA haplotypes (I-a, I-b, II-a, and II-b) among 24 isolates from 11 countries. In a subsequent study, Griffith and Shaw (1998) used mtDNA sequence information to devise a polymerase chain reaction (PCR)-RFLP method to detect Carter's four haplotypes among 90 isolates from Russia and the United Kingdom. At nearly the same time as Carter's work, Goodwin (1991) described by southern blot analysis four haplotypes (A, B, C, and D) among 173 isolates from Mexico, Peru and Europe. In addition, Koh *et al.* (1994) found two other haplotypes (E and F) using Goodwin's method to assay 124 isolates from East Asian countries. In general, the two nomenclature systems exhibit common themes. Carter's haplotype groups I and II seem to correspond to Goodwin's haplotypes A and B, respectively (Carter *et al*., 1990; Goodwin, 1991). It means that Goodwin's haplotype A includes both of Carter's haplotypes I-a and I-b; and Goodwin's haplotype B includes both of Carter's haplotypes II-a and II-b. In addition, haplotypes E and F were included in Carter's haplotype I-b. However, it is not yet explicitly clear how Goodwin's haplotypes C and D.

Adler *et al*. (2004) reported new *P. infestans* pathogen genotypes associated with the *Anarrhichomenum* complex were isolated recently. It was A1 mating type and Ia mitochondrial DNA (mtDNA) haplotype, and therefore differed from the previously described EC-2 lineage, which was A2 and Ic, respectively. Mitochondrial haplotypes of *P. infestans* population had been characterized in many countries in Europe (Cooke *et al*., 2006; Dowley *et al*., 2000; Flier *et al*., 2007; Lebecka *et al.*, 2007; Lehtinen *et al*., 2007; Runno-Paurson *et al*., 2009; Shaw *et al*., 2007), Canada, South and North America (Alder *et al*., 2004; Reis *et al*., 2003; Wangsomboondee *et al*., 2002; Widmark *et al*., 2007), Africa (McLeod *et al*., 2001), and Asia (Ghimire *et al*. 2003; Gotoh *et al*., 2005; Jaimasit and Prakob, 2010).

7. AFLP (Amplified Fragment Length Polymorphism) Fingerprinting

The AFLP method was performed as described for *P. infestans* by Van der Lee *et al*. (1997). The AFLP protocol consisted of four steps: i) primary template preparation in a reaction where genomic DNA was digested with restriction enzymes and simultaneously oligonucleotide adapters were ligated to the restriction fragments; ii) selective pre-amplification of primary template with primers complementary to the adapters and containing one 3' selective nucleotide; iii) selective amplification with the same primers but now fluorescent-labelled at the 5' end and containing two 3' selective nucleotides; and iv) separation of the labelled fragments on polyacrylamide gels.

A total of 158 multilocus AFLP genotypes were identified among the 170 *P. infestans* isolates in the Toluca Valley in 1997. The fungal populations were highly variable and almost every single isolate represented a unique genotype based on the analysis of 165 AFLP marker loci (Flier *et al*., 2003). A total of 69 AFLP genotypes had been detected in 210 isolates collected from France, Norway, Switzerland and United Kingdom during 2001 and 2002 using 137 AFLP loci amplified with two primer combinations. AFLP markers revealed the data that Swiss *P. infestans* subpopulations from potato cv. Santé collected in 2001 differed from the 2002 samples. The UK population showed some similarity to the Swiss population collected in 2001 (Flier *et al*., 2007).

8. Simple Sequence Repeat (SSR) or Microsatellites

Microsatellites are short fragments of DNA in which motifs of 1-6 bases occur in tandem repeats and the molecular marker method used in an investigation to take the advantage of the PCR amplification. Microsatellite sequences are usually characterized by a high degree of length polymorphism, and ideal single-locus co-dominant markers for genetic studies. The amplified locus can be used to determine and discriminate population genetic structure (Cooke and Lees, 2004; Lees *et al*., 2006).

SSR markers are potentially useful tools for identifying *Phytophthora* species and for assessing genetic variation among populations (Garnica *et al*., 2006). Rohner (2002) reported that among 108 isolates of *P. infestans* from Switzerland in 2001 and 2002 only 4 SSR genotypes were detected. On potato, three of the four genotypes occurred at a high frequency, and on tomato only two of the four genotypes were identified. Windmark *et al*. (2007) analyzed 61 Swedish isolates with microsatellite markers, 14 multilocus genotypes had been distinguished based on six polymorphic loci. Lebecka *et al*. (2007) applied 10 SSR markers which were polymorphic in a group of 88 *P. infestans* isolates collected in Poland. There were 55 different genotypes discriminated based on this analysis. SSR markers had been used to analyze for many studies of *P. infestans* (Ben-Jin *et al*., 2009; Brurberg *et al*., 2007; Knapova and Gisi, 2002; Lees *et al*., 2006).

9. Random Amplified Polymorphic DNA (RAPD)

RAPD technique uses as auxiliary tool for the genetic analysis, classification or identification of fungi and provides an unlimited number of markers that can be used in population genetic studies (Abu-El Samen *et al*., 2003; Kim and Lee, 2001). This marker provides DNA polymorphism from different DNA sequences caused by nucleotide pair substitutions, deletions, inversions and translocations (Waugh and Powell, 1992).

A total of 62 *P. infestans* Costa Rican isolates during 1999-2001 had been analyzed by 11 RAPD primers generating 17 RAPD genotypes (Páez *et al*., 2005). Twenty five Turkish isolates had been amplified with 21 RAPD primers indicating that in clade I, US-1 isolates were closest, while US-8 isolates were closest in clade II (Tosun *et al*., 2007). Polymorphic of 80 Chinese isolates had been analyzed by 14

RAPD makers revealing that a total of 189 bands had been amplified with the percentage of polymorphic bands being 95.2% (Ben-Jin *et al*., 2009). A total of 28 Nepalese isolates had been performed with 32 RAPD primers. RAPD markers differentiated isolates of NP1 and NP2 genotypes into three and two RAPD phenotypes, respectively (Ghimire *et al*., 2003). RAPD markers revealed the additional variation within allozyme grouping of Canadian isolates during 1994 and 1995 (Peters *et al*., 2001). The OPG-06 RAPD primer had generated specific patterns for Japanese genotypes US-1, JP-1 and a new A1 (JP-2, JP-3 and JP-4) of *P. infestans* (Akino *et al*., 2008).

10. Sequence Analysis

Sequence analysis is powerful method for phylogenetic relationship between species (Cooke *et al*., 2000) and being applied to gene genealogies or phylogeography within fungal species (Banke *et al*., 2004). DNA sequencing of *P. infestans* had been performed at nuclear or mitochondrial regions. Phylogenetic study of 48 *Phytophthora* species based on nuclear (translation elongation factor 1α and *β*-tubulin) and mitochondrial (cytochrome *c* oxidase subunit 1 and NADH dehydrogenase subunit 1) genes had been performed by Kroon *et al.* (2004). DNA of *P. infestans* had been extracted from 28 historic herbarium samples, amplified and then sequenced at 100 bp fragments from the internal transcribed spacer region 2 revealing that the Ia haplotype of *P. infestans* caused of the late blight epidemics to Irish potato famine in 1845 (Ristaino *et al*., 2001). The P4 region of the mitochondrial genome that contains a portion of the *cox* I gene and the portion of the *ras* gene in nuclear from Ecuadorian isolates had been amplified and sequenced resulting that EC-2 isolates with the Ic haplotype formed a distinct branch in the same clade with *P. infestans* and *P. mirabilis*, *P. phaseoli* and *P. ipomoeae* for both *cox* I and *ras* intron 1 phylogenies and identified as new species, *P. andina*. *Ras* intron 1 sequence data suggest that *P. andina* might have arisen via hybridization between *P. infestans* and *P. mirabilis* (Gómez-Alpizar, 2004; Gómez-Alpizar *et al*., 2008). The P2, P3 and P4 regions of the mitochondrial genome contains portions of *nad*4, *rpl*5 and *cox*1, respectively, were amplified, sequenced and analyzed for historic migration (May and Ristaino, 2004; Ristaino, 2002; Ristaino, 2006).

11. Other Markers

Aggressiveness is determined by using various parameters such as infection frequency, lesion area, incubation period, latent period and sporulation capacity. (Andrivon, 1993; Carlisle *et al*., 2002; Corbiere *et al*., 2010; Flier *et al.*, 2001; Flier *et al.*, 2007; Jaime- Garcia *et al.*, 2000; Lebreton *et al*., 1999; Oyarzun *et al*., 1998; Pliakhnevich and Ivaniuk, 2008; Suassuna *et al*., 2004; Sujkowski, 1986).

Pathogenicity is tested for *P. infestans* isolates by inoculating on different host species. The isolates that can cause disease will be recorded a degree of pathogenicity which is known as virulence. They may attack a wide range of plant species, restricted a number of species or a certain cultivars within a species (Tombolato, 2002). Although *P. infeatans* has universal pathogenicity on potato, not all isolates infect tomato (Chen *et al*., 2008; Chen *et al*., 2009; Kenneth *et al*., 2006; Rohner, 2002). Four different genotypes (US-1, US-8, US-11, and US-17) had been used for pathogenicity test on ten different plant species: potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), petunia (Petunia x hybrida), bell pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), american black nightshade (*Solanum americanum* Mill.), tropical soda apple (*Solanum viarum*), jimsonweed (*Datura stramonium*), cutleaf ground-cherry (*Physalis angulata*), and morning-glory (*Ipomoea* sp.) (Tombolato, 2002). Fontem *et al.* (2005) collected 233 isolates from huckleberry, potato and tomato. The pathogenicity tests showed that the potato, tomato and huckleberry isolates infected their primary hosts as well as the others two plant hosts.

MATERIALS AND METHODS

Phytophthora infestans-Thai Collection

Fungal Isolation and Maintenance

Phytophthora infestans was isolated from a single lesion of naturally infected potato leaf (Appendix A). One hundred thirty two isolates were collected from northern Thailand, Tak and Chiang Mai province, from 2007 to 2009 (Table 3, Appendix Table E1 and Appendix Figure F1-5). The fungi were cultured on rye A agar after a single hyphal tip was transferred.

The US-1 isolate was obtained from W.R. Stevenson, University of Wisconsin-Madison and US-7 and US-11 isolates were obtained from Cornell University via D. Halterman, University of Wisconsin-Madison.

The fungi were maintained on rye A agar for 2 weeks at 18°C in a dark incubator for further studies.

Table 3 Source of *Phytophthora infestans* populations from northern Thailand collected in 2006-2009

Month/Year	Source of isolate			No. of isolate
	Province	District	Sub-District [a]	
Jan/2006	Chiang Mai	Fang	-	4
Jan/2006		San Sai	-	3
Jan/2008			Chedi Mae Krua	37
Feb/2008			Mae Faek Mai	23
Feb/2008			Nong Han	47
Feb/2009		Phrao	Long Khot	5
Dec/2007	Tak	Phop Phra	Ruam Thai Phatthana	13
		Total		132

[a] - = absent data

Chapter 1
Culture Media for *Phytophthora infestans*

1. Culture Media Preparation

The plant ingredients available in Thailand were collected and divided into 2 categories; i) Seed and Dry produces: Seventeen produces in this category included mung bean (MB; *Phaseolus aureus* Roxb.), black and white sesames (B- and W-SS; *Sesamum orientale* L.), red kidney bean (RKB; *Phaseolus vulgaris* L.), job's tear (JT; *Coix lachrymal-jobi* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), peanut (*Arachis hypogaea* Linn.), soybean (SB; *Glycine max* Merr.), dried maize (*Zea mays* L.), sunflower (SFW; *Helianthus annuus* L.), unhusked rice grain (*Oryza sativa* L.), black and white beans (B- and W-B; *Phaseolus vulgaris* L.), Azuki bean (AB; *Vigna angularis* (Willd.) Ohwi & H. Ohashi), common millet (CM; *Panicum miliaceum*) and sorghum (SG; *Sorghum bicolor*), ii) Fresh produces: Kernels, husks and cobs of fresh sweet corn (*Zea mays rugosa*) and waxy corn (*Zea mays ceratina*).

The seed and dry produces were prepared using a slightly modified method for preparing rye A medium (Caten and Jinks, 1968). Sixty grams of each produce was soaked in distilled water with an oxygen generator under dark conditions at room temperature (25°C) for 24 h. The liquid was retained and the swollen or germinated grains were ground in distilled water and incubated at 68°C for 1 h. The slurry was filtered through 3 layers of cheesecloth. The filtered liquid was combined with the retained liquid and used for preparing 1 liter of medium by adding 20 g and 15 g of glucose and agar, respectively, and then, sterilized in the autoclave at 121°C for 15 min, 15 Ib/in^2.

The fresh produces were prepared according to methods used to prepare a pea medium (Bircher and Hohl 1997; Hohl 1991). Two hundred grams of each fresh material was added to 800 ml of distilled water and sterilized at 121°C for 15 min, 15 Ib/in^2. The suspension was filtered and 15 g of agar was added to the supernatant. Twenty grams of glucose were added to the filtrates prepared from corn husks and cobs, but not the filtrates prepared from kernels. All media were adjusted to 1 liter volumes before sterilizing.

The media described above were compared with 6 common media; potato dextrose agar (PDA), oat meal agar (OMA), pea agar (PA), 20% unclarified V8 agar (V8 agar), rye A agar (RA) and rye B agar (RB). A residual of rye grain (rRA) from rye concentrate preparation was also used for culture medium. The preparation of these media are in the Appendix B.

2. Fungal Isolation and Maintenance

Phytophthora infestans was isolated from a single lesion of naturally infected potato leaf. One hundred thirty two isolates were collected from Tak and Chiang Mai province, Thailand. A representative isolate, named CMSS0-06, was used for all experiments. The US-1 isolate was obtained from W. R. Stevenson, University of Wisconsin-Madison and US-7 and US-11 isolates were obtained from Cornell University via D. Halterman, University of Wisconsin-Madison. The fungus was maintained on rye A agar for 2 week at 18°C in a dark incubator for further studies.

3. Media Evaluation

3.1 Mycelial Growth and Sporangial Production

A 5 mm diameter agar plug was taken from the colony margin of *P. infestans* isolate CMSS0-06 and placed in the center of a Petri dish containing 20 ml of media, four replicates (10 Petri dishes/replicate) for each medium. The Petri dishes were placed at 18°C in a dark incubator. The mycelial growth was measured in mm and aerial mycelial growth was assessed visually at 11 days after incubation. After 15 days of incubation, 3 mycelial plugs (0.5 cm) were cut from different positions of the culture plates; one plug obtained from adjoining the original inoculum, one plug between the original inoculum and colony margin, and one plug from the colony margin, then suspended and gently shacked in 1 ml distilled water to obtain a suspension of sporangia. A haemacytometer was then used to determine the number of sporangia from these three plugs and the number of sporangia per cm^2 of the medium surface area was calculated. An average from five Petri dishes was used for each medium. The experiment was repeated twice.

3.2 Induction of Sporangial Production

The selected media supplemented with β-sitosterol were used to induce sporangial production of *P. infestans*-Thai, US-1, US-7 and US-11 isolates. The latter three isolates did not produce sporangia on rye A medium. An agar plug (5 mm diameter) of each isolate was transferred to each selected medium with 0.05 g of β-sitosterol and incubated using the conditions described above. Mycelial growth (mm) was measured after 12 days incubation. Five replicates were used per treatment. Aerial mycelial growth was assessed visually after 15 days incubation. Sporangial production (sporangia/cm^2) on selected media supplemented with β-sitosterol was determined and described previously. Induction of sporangial production on selected media was assessed after 15 days incubation compared with sporulation on rye B agar.

3.3 Oospore Production

The selected media with β-sitosterol supplemented were used to assess the production of oospores. Agar plugs (5 mm diameter) of US-11 (mating type A1) and US-7 (mating type A2) isolates growing on rye B agar were placed 20 mm apart on the selected media and incubated at 18°C in a dark incubator. After three weeks, an average number of oospores was assessed by cutting three agar plugs (3 mm diameter x 4 mm length) from the center of the Petri dish. The agar plugs were placed on glass slide and covered with cover slip, and then, examined for oospores under a light microscope (400X). Three Petri dishes for each selected media were used.

3.4 Fungal Growth Ability

The 132-Thai (collected in Tak and Chiang Mai Provinces), US-1, US-7 and US-11 isolates were compared for growth on selected media. Four agar plugs of each isolate were transferred to each selected medium, three Petri dishes per each isolate. Aerial mycelial growth was assessed visually and compared with rye A and V8 agar after 7 days incubation.

3.5 Long-term Storage

P. infestans isolate CMSS0-06 was selected for this study. The glass vials (Wheaton, screw cap, 16 ml) containing 5 ml-each selected medium were prepared. An agar plug of the isolate was obtained from the colony margin after 14 days incubation and transferred to 80 vials of each medium. The vials were maintained in the dark at 4° and 18°C. At one month intervals, the fungus was transferred from vial to Petri dishes containing rye B agar to determine its viability (colonial growth) and sporangial production as mentioned above using five replications per month.

4. Statistical analyses

All data were analyzed with analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at *P*=0.05. Wilcoxon signed rank test was used to analyze non parameter test at *P*=0.01.

Chapter 2

Morphological Characteristics of *Phytophthora infestans*-Thai Isolates

1. Micro-morphology

1.1 Fungi

One hundred thirty-two of *P. infestans*-Thai isolates culturing on rye A agar were measured on its pedicel length, L:B ratio and oospore diameter. US-7 isolate, mating type A2, was paired with Thai isolates on rye B agar for oospore formation.

1.2 Measurement of Asexual and Sexual Spore

1.2.1 Asexual Spore

The Thai isolates were cultured on rye A agar for 2 weeks at 18^{o}C. The Petri dish of each isolate was filled with 10 ml of distilled water to make a sporangial suspension. The colony surface was gently scraped by L-shaped glass rod. The sporangial suspension was transferred by dropper onto a glass slide and then covered with a cover slip. The sporangia were measured by micrometer. The length (μm) and breadth (μm) of sporangia of each isolate were measured at 100x magnification and subsequently calculated to L:B ratio as following formula;

$$\text{L: B ratio} = \frac{\text{Length of sporangia } (\mu\text{m})}{\text{Breadth of sporangia } (\mu\text{m})}$$

The pedicels of each isolate were measured for length (μm) at 400x magnification. Each isolate, 30 sporangia were measured per experiment.

1.2.2 Sexual Spore

Each Thai isolate was induced to form oospore by pairing with US-7 on rye B agar for 2 weeks. The contact zone of 2 colonies was cut and transferred by cork borer onto a glass slide which had a drop of water and then covered with a cover slip. The cover slip was gently pressed and then, oospores were measured by using micrometer under compound microscope. The dimensions (μm) of oospore were measured at 100x magnification. Each isolate, 30 sporangia were measured per experiment.

1.3 Data Analysis

The isolates were divided into group by size of sporangia (L:B ratio), pedicel and oospore. Size of each morphological characteristics of the isolate was compared using the least significant difference (LSD) test separately.

Chi-square (χ^2) was used for non parameter analysis on relation between size of micro-morphology and source of fungi.

Multi-characteristics of micro-morphologies (group of L:B ratio, pedicel and oospore) were used for classifying into cluster.

The Shannon Index (*H′*) (Shannon, 1948) and Evenness Index (*J′*) (Pielou, 1966) were used for biodiversity analysis of clusters of *P. infestans* Thai isolates classified by multi-characteristic of its morphology.

$$H' = -\sum_{i=1}^{s} pi \ln pi$$

Where; *ni* = The number of individuals in species i

S = The number of species

N = The total number of all individuals

pi = The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: *ni*/*N*

When; *H′* = 0 is low species richness and evenness

H′ = high value of the index is high species evenness and richness

$$J' = \frac{H'}{ln\ s}$$

Where; *H′* = Shannon Index

s = The total number of species in the community

When; *J′* is constrained between 0 and 1.

0 is the less variation in communities between the species.

2. Macro-morphology

2.1 Fungi

One hundred thirty-two of *P. infestans*-Thai isolates were measured on its colonial characteristics on common media; 20% rye A and 20% unclarified V8 agar, and potato tuber slices.

2.2 Media Preparation

See full description in Appendix B for common media preparation.

2.3 Cultural Characteristics of Thai Isolates

Each isolate was cultured on rye A agar, V8 agar and potato tuber slice to determine cultural characteristics after pure culture obtained from infected leaf.

2.3.1 Rye A Agar

A 0.5 cm mycelial plug of each isolate was inoculated at the center of Petri dish containing rye A agar. Two weeks after incubation at 18^{o}C in dark condition, colony type of *P. infestans* culture were determined by visual assessment. Three replications were conducted for each isolate with two repeated experiments.

2.3.2 V8 Agar

A 0.5 cm mycelial plug of each isolate was inoculated at the center of Petri dish containing V8 agar. Ten days after incubation at 18^{o}C in dark condition, growing ability and mycelial mat density were determined by visual assessment. Three replications were conducted for each isolate with two repeated experiments.

2.3.2 Potato Tuber Slices

A 0.5 cm mycelial plug of each isolate was placed in a Petri dish containing moist blotter paper. A potato tuber slice (7 mm thick) of cv. Spunta was placed on the mycelial plug. The potato slice was incubated at 18^{o}C for 7 days. A colony type on tuber slice was determined by visual assessment. Three replications were conducted for each isolate with two repeated experiments.

2.4 Data Analysis

The isolates were divided into group by culture characteristics on potato tuber slices and rye A and V8 agar.

Multi-characteristics of macro-morphologies (colonial type on tuber slices, rye A and V8 agar) were used for classifying into cluster.

The Shannon Index (*H′*) (Shannon, 1948) and Evenness Index (*J′*) (Pielou, 1966) were used for biodiversity analysis of clusters of *P. infestans* Thai isolates classified by multi-characteristic of macro-morphology.

Chapter 3

Pathogenicity and Aggressiveness of *Phytophthora infestans* Isolates

1. Pathogenicities of Thai Isolates on Primary and Alternative Host

1.1 Fungi

One hundred thirty-two, 131, and 130 of *P. infestans*-Thai isolates were used for pathogenicity experiment on potato leaflet, potato tuber and tomato leaflet, respectively.

1.2 Testing Host

1.2.1 Potato and Tomato Leaflets

Two cultivars of potato leaflets (*Solanum tuberosum* L.), cv. Spunta and cv. Atlantic, were used for study. Two cultivars of tomato leaflets (*Solanum lycopersicum* L.) were cv. Seeda which was cherry tomato and cv. Delta which was table tomato using for study. Potato and tomato leaflets were prepared for pathogenicity test using detached leaf technique.

1.2.2 Potato Tuber Slices and Discs

Two cultivars of potato tubers (*Solanum tuberosum* L.) cv. Spunta and cv. Atlantic without fungicide treatment were bought from local market and farmer using for study.

1.3 Pathogenicity Test

1.3.1 Potato and Tomato Leaflets

Fully expanded leaflets were chosen from plants between 6 weeks old or initiation of flowering stage, flower buds were removed. Leaflets from the third to the fifth position of shoot were selected. Leaflets were washed with sterile distilled water for about 10 min, and blotted slightly on paper towels to remove excess moisture. The leaflets were placed abaxial side up into 9 cm moist Petri dishes, each containing 2 to 3 layers of moist sterile blotting paper. Sporangial suspension, 10^4 spore/ml, was inoculated by placing one 10-µl drop at the middle of the midrib of each leaflet. The chambers were incubated at 18^{o}C in dark condition for 7 days. Six leaves were determined for each isolate.

1.3.2 Potato Tuber Slices and Discs

Surfaces of potato tubers were sterilized in 1% sodium hypochlorite for about 5 to 10 min and cut into (i) slices that were 0.5 to 1 cm thick and cross surface dimension of potato tuber used for lesion area determination, 3 slices were determined for each isolates, and (ii) discs that were 0.7 x 0.5 cm (diameter x thick) of potato tuber used for determination of infection frequency (3 replicates per sample, 3 discs per replicate) and sporulation capacity (3 discs per sample) for each isolates.

The potato slices and discs were placed on moist blotter paper in a Petri dish and each slice or disc was inoculated with a 10-µl droplet of sporangial suspension containing 10^4 spore/ml. The chambers were incubated at 18^oC in dark condition for 7 days.

1.4 Pathogenicity Determination

After 7 days of incubation, fitness parameters were measured comparatively in the isolates using the method of Tooley *et al.* (1986): (i) infection frequency, IF, the proportion of inoculated leaflets or tuber discs infected; (ii) lesion area, LA, the area of lesion produced 5 days after inoculation on detached leaflets or tuber slices; (iii) sporulation capacity, SC, the number of sporangia produced per square centimeter of lesion in intact leaflets or tuber slices.

Firstly, IF was recorded by using a stereomicroscope to determine the proportion of leaflets and discs showing fungal sporulation. Secondly, LA was measured in infected leaflets and tuber slices by measure the lesion size and finally converted to percent of lesion area compared to the control. Lastly, SC was measured as follows. Three infected leaflets placed in a beaker with 10 ml of sterile distilled water and shaken for 2 min to dislodge sporangia. Sporangia were counted by using a haemacytometer (3 replicates per sample, 3 counts per replicate). Sporulation capacity per square centimeter of lesion area was calculated. Three infected tuber discs placed in a vial with 1 ml of sterile distilled water and shaken for 2 min to dislodge sporangia. Sporangia were counted by using a haemacytometer (3 replicates per sample, 3 counts per replicate).

1.5 Data Analysis

The isolates were divided into groups by value of fitness parameter; IF, LA and SC, using frequency distribution. Mean of IF, LA and SC value of the isolates were compared using the Least Significant Difference (LSD) separately. Means severity on types/cultivars of infected host were compared using the Least Significant Difference (LSD). Chi-square (χ^2) was used for analysis on relationship between groups of fitness parameter and sources of the isolates. All statistical analyses were performed using the SPSS version 11.5.

2. Host Range Test of *Phytophthora infestans* Thai-isolates

Solanaceous crops cultivated in Thailand and weed plants found in potato field were used for pathogenicity test with representative *P. infestans* Thai-isolate which was isolate 39, producing large lesion on both potato leaflets cv. Atlantic and Spunta by using detached leaf method.

2.1 Plants and Experiments

Eleven solanaceous crops including horticultural and ornamental plants and weeds such as potato leaflet (*Solanum tuberrosum*) cv. Atlantic, pea eggplant leaf (*Solanum torvum* Swartz.), eggplant leaf and fruit (*Solanum melongena* L.), Thai green eggplant leaf (*Solanum xanthocarpum* Schrad. & Wendl.), tobacco leaf (*Nicotiana tabacum* Linn.), chilli pepper leaf and fruit (*Capsicum annuum* Linn.), tomato leaf and fruit (*Solanum lycopersicum* L.) cv. Seeda, petunia leaf (*Petunia* sp.), night-blooming Jessamine leaf (*Cestrum nocturnum* L.), yesterday-today-and-tomorrow leaf (*Brunfelsia hopeana* Benth.), thorn apple (*Datura metel*) and ground cherry (*Physalis angulata* L.) and one convolvulaceous plant, water morning glory (*lpomoea aquatica* Forsk.), were used to test.

For all experiments, fully-expanded leaves or leaflets at the upper third of the plant and green mature of fruits were detached for inoculation. Leaves or leaflets or fruits were surface-sterilized in 0.5% NaOCl for 5 min, rinsed in sterile water, and dried on sterile blotting paper. Those plant leaves were placed, abaxial leaf surface face-up, in a 9-cm Petri dish or a plastic box moisture chamber which contained 3 layers of moist blotter papers. Twenty microliters of spore suspension (10^4 spore/ml) were dropped on leaves or leaflets midrib and fruit stem end. Inoculated plants, as well as controls (water inoculated plants), were incubated at 18^oC for 14 days. Six leaves or leaflets or fruits were used for one experiment. Two separate plant inoculation experiments were performed.

2.2 Assessment

An 'Incubation Period' which was the period between inoculation and symptom expression was recorded. The incubation period was observed daily by visual examination and recorded after a lesion sized at ≥ 0.5 cm of diameter. The number of infected plants was also recorded.

Chapter 4
Genetic Distribution and Characteristics of *Phytophthora infestans*-Thai Isolates

1. Mating Type of *Phytophthora infestans*-Thai Isolates

1.1 Fungi

One hundred thirty-two of *P. infestans*-Thai isolates were determined for mating type A1, A2 or self-fertile. The US-1 and US-7 isolates are mating type A1 and A2, respectively, were used for known tester.

1.2 Mating Type Test for Thai Isolates

An agar plug, 0.5 cm diameter, of an unknown mating-type isolate, Thai isolates, were placed on one side of a Petri dish containing rye B agar with a plug of a known mating type A1 isolate on the other side. A known A2 isolate was followed a same as mentioned earlier in another Petri dish. A pair of agar plugs of each Thai isolate was also placed on same Petri dish for self-fertile test. The Petri dishes were incubated at 18^{o}C for 2 weeks. The contact margin of colonies was observed for oospore production under a compound microscope. The experiment was 3 replicates for the isolates tested.

When an unknown mating-type isolate forms oospores in the Petri dish with the A1 isolate, the unknown isolate is an A2 isolate and when the isolate form oospores with the A2 isolate, the unknown isolate is an A1 isolate. When an unknown isolate forms oospores with a same isolate are considered to be self-fertile.

2. Metalaxyl Sensitivity of *Phytophthora infestans*-Thai Isolates

2.1 Metalaxyl Sensitivity Test

2.1.1 Fungi

One hundred thirty-two of *P. infestans*-Thai isolates were treated with phenylamide fungicide, metalaxyl. The US-1, US-7 and US-11 isolates were used as positive control; sensitive, resistant and resistant isolates, respectively.

2.1.2 Experiments

A mycelial plug, 0.5 cm, of each isolate was transferred to rye A agar supplemented with 25% commercial grade metalaxyl (Penraxyl, PentaCheMee Co. Ltd, Thailand) at concentration of 0, 5 and 100 µg/ml metalaxyl at the center of each Petri dish.

Metalaxyl was prepared from commercial grade to 20 mg/ml of stock solution in pure dimethylsulfoxide (DMSO) and appropriate volumes were added to molten agar (50^{o}C) after autoclaving to bring it to the desired concentrations (Appendix C). An amount of DMSO without metalaxyl was added to the control plate. Three replications (4 plates per replicate) were conducted per treatment for each isolate. The Petri dishes were maintained at 18^{o}C in the dark. Radial growth of all treatments were measured when the colony in the control reached the plate margin and calculated as a percentage of growth on non-amended rye A medium (control plate).

Isolates with a colony diameter less than 40% of the non-amended control at 5 and 100 μg/ml were classified as metalaxyl sensitive. Isolates with colony diameter greater than 40% of the non-amended control at 5 μg/ml but less than 40% of the non-amended control at 100 μg/ml were classified as metalaxyl intermediate. Isolates with colony diameter greater than 40% of the non-amended control at both 5 and 100 μg/ml were classified as metalaxyl resistant (Deahl *et al*., 1995)

2.1.3 Data Analysis

Chi-squared (χ^2) analyses were used to test for analysis on relationship between metalaxyl sensitivity and source of isolate, and between metalaxyl sensitivity and size of reproductive propagules (Chapter 1).

2.2 The Purity of Metalaxyl Used in Metalaxyl Sensitivity Test

2.2.1 Fungi

Seven representative Thai-isolates; CMSS1-39, CMSS2-17, CMSS3-04, CMPr4-05, TKPP1-01, TKPP1-02, and TKPP1-03 were selected by source of isolates and its sensitivity to metalaxyl (the experiment 2.1) for this study. Three US-isolates were also used.

2.2.2 Experiments

The comparative test on sensitivities of *P. infestans* to metalaxyl technical (98.1% a.i.; Sharp Formulators Co. Ltd., Thailand) and commercial (25% a.i.; Penraxyl, PentaCheMee Co. Ltd, Thailand) grade amended in rye A agar were conducted. Metalaxyl technical grade were prepared to 100 mg/ml of stock solution (Appendix C1-C2) and then, diluted to the desired concentration. Method to test was mentioned as above. Three replications (4 plates per replicate) were conducted per treatment for each isolate.

2.2.3 Data Analysis

Wilcoxon Signed Ranks were used to differentiate analyze between technical and commercial grade amended media.

3. Mitochondrial DNA Haplotypes of *Phytophthora infestans*-Thai Isolates

3.1 Fungi

One hundred thirty-two of *P. infestans*-Thai were characterized for mtDNA haplotypes which distinguished by the RFLP-PCR method.

3.2 Extraction of Total DNA

Mycelium of *P. infestans* was produced on corn A agar for 2 weeks, when the aerial mycelia were scraped off from the Petri dish with a spatula. An extraction protocol was modified from Mahuku (2004), approximately 150 mg of mycelia were ground with a pestle in a mortar containing 1500 μl of TES extraction buffer (0.2 M Tris-HCl [pH 8], 10 mM EDTA [pH 8], 0.5 M NaCl, 1% SDS) with sterile sand. The mycelium was macerated for 2 min until milky, transferred to 1.5-ml microcentrifuge tube (1st microcentrifuge tube), and then vigorously mixed for 2-3 min using vortex mixer. Five hundred microliters of TES buffer and 20 μl of proteinase K (final concentration of 50 μg/ml) were added into the mixed tube. The tube was thoroughly mixed and placed in a water bath at 65^{o}C for 30 min. The incubated tube was centrifuged at 16,000 xg for 15 min. The supernatant was transferred to a new tube (2nd microcentrifuge tube), subsequently added 1.5 volume of 7.5 M ammonium acetate and then thoroughly mixed by vortexing for 2-3 min. The tube was centrifuged at 16,000 xg for 15 min after incubating on ice (or at approximately -5^{o}C in refrigerator) for 15 min. The supernatant was transferred to a new tube (3th microcentrifuge tube), subsequently added an equal volume of ice-cold isopropanol, and then gently mixed by inverting the tube for about 10 times. The tube was incubated at -20^{o}C for 1-2 h before centrifuging at 16,000 xg for 20 min to retrieve a

DNA pellet. After decanting the supernatant, the pellet was gently washed with 500 μl of 70% ethanol which were then poured off. The tube was turned upside-down on clean sterile paper towels for 5-10 min to air-dry DNA. The DNA pellet was eluted with twice-repeated extractions with 100 μl of sterile reverse osmosis (RO) water, each time centrifuging at 16,000 xg for 15 min to avoid collecting pelleted polysaccharides. The DNA solution was transferred to a new tube (4^{th} microcentrifuge tube), subsequently added 2 μl of 40 mg/ml RNase A, and then incubated at 37^{o}C for 60 min.

3.3 Quantitative Estimation of DNA Concentrations

The isolated DNA was measured by using the Nano-Drop (ND-1000; NanoDrop Technologies, Welmington, USA) or NanoVette (DU®730; Beckman Coulter Inc., Fulllerton, CA, USA) spectrophotometer. DNA concentrations were confirmed using agarose gel electrophoresis. Ten microliters of purified DNA was run on a 1.5% (w/v) agarose gel containing 0.1 μg/mL of ethidium bromide. DNA was visualized using a UV transilluminator model TM-26 (Labnet International, Inc.).

3.4 RFLP-PCR for mtDNA

The mtDNA haplotypes were clear identified into four types; Ia, IIa, Ib and IIb which were have been described by Carter *et al.* (1990) using RFLP technique. Subsequently, *P. infestans* four polymorphic regions in the mitochondrial genome; known as P1, P2, P3 and P4, were determined according to the method of Griffith and Shaw (1998) using RFLP-PCR. The P2 and P4 portions were amplified by PCR using primer pairs as shown in Table 4 and giving product length of 1,240 and 964 bp, respectively, and followed by the digestion with restriction enzymes *Msp*I and *Eco*RI, respectively.

Table 4 Oligonucleotide primers used for amplification of polymorphic mitochondrial DNA regions P2 and P4

Locus	Primer sequence	Primer length (bp)	Product length (bp)
P2	F2: 5’-TTC CCT TTG TCC TCT ACC GAT-3’	21; 34,081-34,101	1,243
	R2: 5’-GCT TAT GCT TCA GTT GCT CAT-3’	21; 35,323-35,303	
P4	F4: 5’-TGG TCA TCC AGA GGT TTA TGT T-3’	22; 9,329-9,350	964
	R4: 5’-CCG ATA CCG ATA CCA GCA CCA A-3’	22; 10,292-10,271	

3.5 PCR-RFLP Procedure

3.5.1 An Optimum of PCR Profile

PCRs with a model MultiGene Gradient Thermal Cycler TC9600-G (Labnet International, Inc.) were optimized to maximum yield of the desired PCR product and reduce levels of nonspecific products. An optimum amount of DNA template, temperature of annealing in PCR, and a sensitivity of primer were modified (Appendix D).

3.5.2 PCR Conditions

In this research, PCRs were performed with a Thermal Cycler model MultiGene Gradient TC9600-G (Labnet International, Inc.). The genomic DNA mixed with PCR master mix in 200-μl thin wall PCR tube.

Amplification was as follows for both primer combinations (final concentrations): 0.625 μl of genomic DNA (50 ng/μl) was added to a total volume of 25 μl containing 2 μl of 2.5 μM of each deoxynucleoside triphosphates (dNTPs), 1 μl of 25 mM of $MgCl_2$, 0.33 μl of 10 μM of P2-oligonucleotide primer (Bio Basic, Inc.) or 0.26 μl of 10 μM of P4-oligonucleotide primer (Bio Basic, Inc.), 1.5 μl of 10x Thermo buffer (500 mM KCl and 100 mM Tris-HCl, pH 8.8), and 0.125 μl of 5U/μl of *Taq* DNA polymerase (Fermentas) and then adjusted to final volume with sterile RO-H_2O.

The PCR conditions as following,

Primer pair of P2: Thermal cycling parameters were initial denaturation at 94^{o}C for 90 s, followed by 35 cycles consisting of denaturation at 94^{o}C for 40 s, annealing at 62 for 60 s, and extension at 72^{o}C for 60 s. A final extension at 72^{o}C for 5 min followed.

Primer pair of P4: Thermal cycling parameters were initial denaturation at 94^{o}C for 90 s, followed by 35 cycles consisting of denaturation at 94^{o}C for 40 s, annealing at 55 for 60 s, and extension at 72^{o}C for 60 s. A final extension at 72^{o}C for 5 min followed.

3.5.3 Restriction Enzyme Digestion

Four microliters of the amplified DNA was digested either with 0.1 μl of 10 U of the restriction enzyme *Msp*I (Promega) for region P2 or with 0.1 μl 10 U of *EcoR*I (Promega) for region P4 in a 20-μl volume restriction digest containing 2 μl of 10x restriction enzyme buffer [(L buffer), 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithioerythrit_(Promega)], 0.2 μl of 10 mg/ml acetylated BSA, and adjusted with DEPC treated water at 37^{o}C for 12 to 24 h and then at 65^{o}C for 10 min.

3.5.4 Agarose Gel Electrophoresis

One hundred grams of 2% agarose solution for gel electrophoresis of digested PCR products were prepared by putting 2 g of agarose powder into a flask, adding with 100 ml of electrophoresis buffer (0.5X TAE) and then heating in a microwave oven until completely melted. After cooling the agarose solution to about 60^{o}C, it was poured into a casting tray containing a sample comb and allowed to solidify at room temperature. The comb was removed after the gel solidified. The gel, still in the tray, was immersed in electrophoresis buffer which was filled in the electrophoresis chamber model DYCP-34A (Beijing Liuyi Instrument Factory) or GelMate 2000 (Toyobo).

3.5.5 The Electrophoresis of Digested Product

Ten microliters of digested DNA samples were mixed with 2 μl of 6X loading dye and loaded onto a 2% agarose gel. The gel was run at 150 V for 60 min, and then stained with ethidium bromide. A 100-bp DNA ladder was included in each gel as a molecular size standard. Restriction patterns were visualized with a UV transilluminator model TM-26 (Labnet International, Inc.) at 302 nm and then images were recorded by a digital camera (PowerShot A640, Cannon).

4. DNA Sequencing

4.1 Sampling and DNA Extraction

An about sixty Thai isolates and 7 US isolates; US-8 3.7, US-8 BB, US-8 2125, US-1 US940501, US-1 94051, 126-3-18 ND, US-11 US980008 were used in this study. Total genomic DNA was extracted from mycelium as above protocol (3.2).

4.2 PCR

Three portions of nuclear gene were amplified via polymerase chain reaction (PCR). A 223-bp intron (Intron Ras) located in the 5'untranslated region of the gene *Ras*, a 600-bp portion (Ras) covered part of exon 3, exon 4, exon 5, part of exon 6 and intron 3 to 5 (Appendix Figure F6) and a 542-bp amplified portion of B-Tubulin, excluding intron. The PCR primers are shown in Table 5.

A 20-μl reaction was carried out per primer and isolate. Each reaction contained 10 μl of 2X PCR Master Mix (Promega, WI, USA) containing *Taq* DNA

Polymerase, dNTPs, $MgCl_2$ and reaction buffers, 0.5 μl of 10 μM each forward and reverse primer, 8.5 μl of Nuclease-Free Water and 0.5 μl of 25 μg/μl DNA template.

Table 5 Primers used for nucleotide sequencing of *Phytophthora infestans*

Nuclear	Primer	Primer sequence	Primer length (bp)	Primer position
Intron Ras[a]	IRF	5' TTG CAG CAC AAC CCA AGA CG 3'	20	442-461
	IRR	5' TGC ACG TAC TAT TCG GGG TTC 3'	21	768-789
Ras[a]	RASF	5' CGT GTC TGC TTC TCC GTT TCG 3'	21	916-936
	RASR	5' CCA GGC TTT CGG CAA ATT CC 3'	20	1496-1515
B-Tubulin[b]	TUB901	5' TAC GAC ATT TGC TTC CG 3'	17	901-918
	TUB1401	5' CGC TTG AAC ATC TCC TGG 3'	18	1383-1401

[a]Gomez *et al*. (2003)

[b]Gomez-Alpizar *et al*. (2007)

Cycling conditions were 96°C (1 min); then 35 cycles of 96°C (1 min), 52°C (1 min), 72°C (2 min); and a final extension of 72°C (10 min). Amplifications were performed in a Techne TC-512 Thermal Cycler (Bibby Scientific Limited, Staffordshire, UK) or Mastercycler EP Gradient (Eppenddorf, NY, USA).

Five microliters of the product was loaded onto 1% agarose gel electrophoresis to determine the quality. The PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA).

4.3 PCR product sequencing

Sequencing of PCR products was carried out in 10 μl reaction volumes in 96 well plates or tubes. For each PCR product, forward and reverse sequencing reactions were performed. To 1 μl forward or reverse primer (10 μM), 1 μl BigDye® Terminator v3.1Cycle Sequencing Kit (Applied Biosystem, Foster City, CA), 1.5 μl 5X BigDye Buffer and 2.5 μl sterile deioninzed water was added to purified PCR product (4 μl).

Thermocycling was performed on a Techne TC-512 Thermal Cycler (Bibby Scientific Limited, Staffordshire, UK) or Mastercycler EP Gradient (Eppenddorf, NY, USA). Following an initial activation step of heating to 95^{o}C for 180 seconds, were 50 cycles of denaturation at 96^{o}C for 15 seconds, annealing at 58^{o}C for 240 seconds and extension at 72^{o}C for 7 minutes.

4.4 Sequencing clean up with CleanSeq magnetic beads

The cooled (4^{o}C) CleanSEQ® beads (Beckman Coulter, MA, USA) were mixed by flicking bottle with fingers. A 10 μl of beads and 62 μl of freshly prepared 85% ethanol were added to each sequencing template and mixed by pipette up and down 7 times. The plate of sequencing templates was placed on magnetic plate for 3 min, to clear the solution. The plate or tubes on the magnetic plate was slowly withdrew the liquid and then, placed on non magnetic plate. One hundred microliters of 85% ethanol were added and allowed to sit for 30 sec. The plate of sequencing templates was placed on magnetic plate for 3 min, to clear the solution, and withdrew as much liquid as possible. Forty microliters of sterile deionized water were added and incubated for 5 min. After 3 min on the magnetic plate, 20 μl of the supernatant was transferred to new plate. Double-strand sequencing of DNA was performed using 3730xl DNA Analyzer (Applied Biosystems) at the University of Wisconsin-Madison Biotechnology Center sequencing facility.

4.5 Sequence analysis

Sequences were aligned manually and edited with DNASTAR® (DNASTAR Inc., WI, USA). Multiple sequence alignments were done using Clustal X.

5. Random Amplification of Polymorphic DNA (RAPD) Fingerprinting

5.1 Sampling and DNA Extraction

An about 45 Thai isolates and 2 US (US-1 and US-11) isolates were used in this study. Total genomic DNA was extracted from mycelium as above protocol (3.2).

5.2 RAPD analysis

RAPD patterns were generated for 45-Thai and 2-US isolates, which were representative population and randomly selected. Seventeen random primers were selected with distinct amplified bands, high polymorphism and stability from 42 random primers synthesized by Operon technologies (Alameda, CA, USA), Invitrogen (Carlsbad, CA, USA) and Ghimire *et al*. (2003). The primer sequences are shown in Table 6.

Table 6 RAPD primers

Sequence (5'-3')	RAPD primer	Source
GGC TGC GAC A	OPY-5	OPERON
AGA GCC GTC A	OPY-7	OPERON
CCA GAT GCA C	OPE-3	OPERON
GGT GAC GCA G	OPB-07	OPERON
CCT TGA CGC A	OPB-12	OPERON
AGG GAA CGA G	OPB-17	OPERON
GTG AGG CGT C	OPC-02	OPERON
ACG ACC GAC A	BA97	INVITROGEN
AGT CGG GTG G	S11	INVITROGEN
CCT GGG TGG A	-	Ghimire *et al*., 2003
CCG GCC CCA A	-	Ghimire *et al*., 2003
GTC CCA GAG C	-	Ghimire *et al*., 2003
GGG CAC GCG A	-	Ghimire *et al*., 2003
GAG GGC GAG G	-	Ghimire *et al*., 2003
GAG CAC CAG G	-	Ghimire *et al*., 2003
GAG CAC GGG G	-	Ghimire *et al*., 2003
GGG CGC GAG T	-	Ghimire *et al*., 2003

DNA amplification was performed in a Thermal Cycler model MultiGene Gradient TC9600-G (Labnet International, Inc.) with one cycle at 94°C for 3 min, followed by 35 cycles at 94°C for 45 sec, 35°C for 1 min and 72°C for 1 min, and a final cycle at 72°C for 7 min. Reactions were carried out in 25 μl volumes containing 1X DNA polymerase buffer (50 mM Tris-HCl (pH 8.5), 2 mM $MgCl_2$, 50 mM KCl, 0.1 Triton X-100), 0.2 mM of each dNTP, 0.4 μM of primer, 1 U of *Taq* DNA polymerase and 2 μl of genomic template DNA (25 ng/μl).

DNA amplification products were separated in 1.8% agarose gels at a in 0.5X TAE buffer. The DNA band patterns were visualized with a UV transilluminator model TM-26 (Labnet International, Inc.) at 302 nm and then images were recorded by a digital camera (PowerShot A640, Cannon).

5.3 Data analysis

The products were scored manually and analyzed as binary data, with 1 and 0 representing the presence and absence of bands, respectively. The genetic distance and genetic identity between isolates were calculated using NTSYS-PC 2.0 software.

RESULTS

Chapter 1
Culture Media for *Phytophthora infestans*

1. Mycelial Growth, Sporangial Production, and Aerial Mycelial Production

When the selected *P. infestans*, CMSS0-06, was cultured on different culture media, the radial growth varied from slight to vigorous colony expansion (Table 7). The isolate grew on all media with the largest colony diameter on rRA medium. Colony growth was somewhat less on media containing RKB, SFW, 5% RA, MB, and BSS. The smallest radial growth was obtained on CM medium.

The production of limoniform, semipapillate sporangia typical of the selected isolate, CMSS0-06, was observed on the 33 media and ranged from 83 to 3,424 sporangia/cm^2 (Table 7). BB medium supported the greatest production of sporangia and produced an average of 3,424 sporagia/cm^2, whereas the lowest sporulation was found on CM medium. Other media which supported good sporulation were WSS, SFW, 20% RA, SB and BSS media at 2,776, 2,155, 1,952, 1,757 and 1,750 sporagia/cm^2, respectively.

BSS, AB, RKB, BB and SFW media supported abundant aerial mycelial production (Table 7). Very sparse mycelial growth was observed on rRA, 5% RA and MB media. Mycelial growth and sporangial production were satisfactory on SB and SFW media, but these media were rather thick in consistency and not completely dissolved.

The BB, RKB, BSS and SFW media using locally available substrates were selected for further study based on radial growth, intensity of sporulation and mycelium growth, and ease of preparation.

Table 7 Mycelial growth and aerial mycelial intensity at 11 days (A) and sporangial production at 15 days (B) of *Phytophthora infestans* isolate CMSS0-06 on media prepared from grains and fresh produces compared with common media

Media	Abbreviation	Growth (mm)	Aerial mycelium[a]	Spore (spore/cm^2 of surface)[b]
Munbean	MB	83 b	++	858 i
Azuki bean	Az	75 d	++++	997 hi
Black bean	BB	68 e	++++	3,424 a
White bean	WB	77 cd	++	1,081 hi
Red kidney bean	RKB	85 b	++++	1,479 efg
Peanut	PN	77 cd	++	488 jk
Soybean	SB	68 e	+++	1,757 de
White sesame	WSS	63 fgh	++	2,776 b
Black sesame	BSS	83 b	++++	1,750 de
Sorghum	SG	49 j	+	160 mn
Common millet	CM	14 m	+	83 n
Job's tear	JT	49 j	+	788 i
Dried maize	DM	56 i	+	1,235 gh
Unhusked rice grain	UR	74 d	+	167 lmn
Sunflower	SFW	85 b	++++	2,155 c
Barley	BL	44 j	+	174 lmn
Wheat	Wh	81 bc	+	223 jklmn
Sweet corn kernel	SCK	64 ef	+++	1,590 ef
Sweet corn cob	SCC	73 d	++	279 jklmn
Sweet corn husk	SCH	59 ghi	+	1,632 ef
Waxy corn kernel	WCK	63 fgh	++	1,360 fg
Waxy corn cob	WCC	74 d	++	502 j
Waxy corn husk	WCH	58 hi	+	467 jklmn
5% rye A	5% RA	83 b	++	174 lmn
10% rye A	10% RA	66 ef	+++	474 jkl
15 % rye A	15% RA	48 j	+++	893 i
20% rye A	20% RA	75 d	++++	1,952 cd
r Rye A	rRA	90 a	+	195klmn
rye B	RB	59 ghi	+++	830 i
V8	V8	64 fg	++++	1,534 ef
Potato dextrose agar	PDA	30 l	++	286 jklmn
Pea agar	PA	37 k	+	300 jklmn
Oat meal agar	OMA	56 i	++++	823 i

[a]Aerial mycelium; + = very sparse, ++ = sparse, +++ = moderate, ++++ = abundant.

[b]Values followed by the same letter for each column are not significantly different at *P* = 0.05 according to Duncan’s new multiple range test.

Use of fresh products resulted in clear media. Media prepared from sweet corn kernels without glucose supported growth, sporulation, mycelial intensity and were easy to prepare.

2. Sporangial Induction

The US isolates used in the experiments produced fewer sporangia on the common medium, rye A, but sporulated on rye B medium. Thai and US isolates were used to determine if sporulation would improve if selected media are supplemented with β-sitosterol. CMSS0-06 grew well and showed an abundance of sporangia and aerial mycelium on all selected media except on SFW B medium. US-1 isolate exhibited vigorous growth, but limited sporulation on BB B medium and no sporulation on RKB B medium. US-7 had less growth on all selected media and no sporulation on BB B medium (Table 8). Selected media except SFW B supported growth of US-11 (Table 9).

3. Oospore Production

The number of oospores of *P. infestans* US-7 and US-11 produced on RB, BB B and RKB B media is shown in Table 10. The addition of β-sitosterol to RKB significantly improved oospore production. Even though the mycelia were sparse on RKB B medium, the isolated produced a large number of oospores. No oospore formation was observed on BSS B and SFW B media.

4. Growth ability

One hundred thirty five isolates were tested for growing ability on selected media. *P. infestans* showed difference in growth ability and mycelia mat intensity on the selected media. Growth ability on sunflower, black bean, red kidney bean, black sesame and V8 media had been divided into 5, 4, 3, 4, and 3 levels, respectively. Thirty three percent and 3 percent showed no growth on SFW and V8 media, respectively. Almost all isolates showed a high growth rate on BB, RKB, and BSS media, but a few isolates showed good growth on SFW medium (Fig. 3A). Mycelial growth of all 135 isolates was compared on each medium after 7 days incubation. Sparse mycelium was observed on SFW medium whereas growth on another 4 media was abundant (Fig. 3B).

5. Long-term storage

P. infestans was successfully recovered after 8 months in storage at 4° and 18°C from vials containing RA, BSS, BB, SFW and RKB (Table 11).

Isolates could still produce numerous sporangia throughout 8 months storage (Table 11). After 1 month storage, the fungal cultures which were maintained in the vials containing RKB stored at 4°C and RA stored at 18°C produced abundant sporangia. After 8 months storage, sporangial production by fungi recovered from all media maintained at 4° and 18°C storage was slightly less than isolates tested after 1 and 5 month (s) storage. The mean number of sporangia from all media after 8 months storage between 4° and 18°C were compared and showed no significant differences in sporulation (Table 11).

Table 8 Mean of mycelial growth and sporangial induction of US-1 and US-7 isolates on 5-selected media supplemented with β-sitosterol

Medium[a]	Isolate of *Phytophthora infestans*[b, c]					
	US-1			US-7		
	radial growth[d]	sporangia[e]	aerial mycelium[f]	radial growth[d]	sporangia[e]	aerial mycelium[f]
RB	83.0 a	1.9×10^3 a	++++	78.0 a	4.7×10^3 ab	+++
BSS B	27.0 d	1.4×10^3 c	+	32.0 b	0.0 ab	++
BB B	60.0 b	4.7×10^3 b	++	31.0 bc	4.2×10^3 a	++
SFW B	58.0 e	1.1 x 104 c	++	32.0 b	5.6×10^3 b	++
RKB B	23.5 c	0.0 c	+	30.0 c	6.1×10^3 a	++

[a]RB = rye B agar, BSS B = black sesame B agar, BB B = black bean B agar, SFW B = sunflower B agar and RKB B = red kidney bean B agar were supplemented with 0.05 g of β-sitosterol

[b]US-1, US-7 and US-11 were American isolates. CMSS0-06 was Thai isolate

[c]Values followed by the same letter for each column are not significantly different at $P = 0.05$ according to Duncan's new multiple range test

[d]Each figure is average radial growth (in mm) after 11 days incubation

[e]Each figure is average no. of sporangia (sporangia/cm^2) after 15 days incubation

[f]Determined after 15 days incubation: + = very sparse, ++ = sparse, +++ = moderate, ++++ = abundant

Table 9 Mean of mycelial growth and sporangial induction of US-11 and CMSS0-06 isolates on 5-selected media supplemented with *β*-sitosterol

Medium[a]	Isolate of *Phytophthora infestans*[b, c]					
	US-11			CMSS0-06		
	radial growth[d]	sporangia[e]	aerial mycelium[f]	radial growth[d]	sporangia[e]	aerial mycelium[f]
RB	90.0 a	5.8×10^3 a	+++	78.7 a	9.0×10^4 ab	++++
BSS B	74.2 b	1.4×10^3 b	+	64.3 b	8.9×10^3 cd	++++
BB B	85.3 a	2.4×10^3 ab	++++	54.0 c	3.2×10^4 a	++++
SFW B	27.5 d	3.8×10^3 b	+	19.3 d	1.1×10^5 d	+++
RKB B	53.0 c	2.4×10^3 b	+	63.0 b	5.8×10^4 bc	++++

[a]RB = rye B agar, BSS B = black sesame B agar, BB B = black bean B agar, SFW B = sunflower B agar and RKB B = red kidney bean B agar were supplemented with 0.05 g of *β*-sitosterol

[b]US-1, US-7 and US-11 were American isolates. CMSS0-06 was Thai isolate

[c]Values followed by the same letter for each column are not significantly different at $P = 0.05$ according to Duncan's new multiple range test

[d]Each figure is average radial growth (in mm) after 11 days incubation

[e]Each figure is average no. of sporangia (sporangia/cm^2) after 15 days incubation

[f]Determined after 15 days incubation: + = very sparse, ++ = sparse, +++ = moderate, ++++ = abundant

Table 10 Oospore production of *Phytophthora infestans* mating between US-7 and US-11 isolates on 5 selected media after three weeks incubation

Medium[a]	No.of oospore[b, c]
rye B agar	887 b
black sesame B agar	0.0 d
black bean B agar	345 c
sunflower B agar	0.0 d
red kidney bean B agar	1,324 a

[a]Media were supplemented with 0.05 g of β-sitosterol

[b]Each figure is an average number of oospores (3x4 mm of agar plug)

[c]Values followed by the same letter for each column are not significantly different at P = 0.05 according to Duncan's new multiple range test

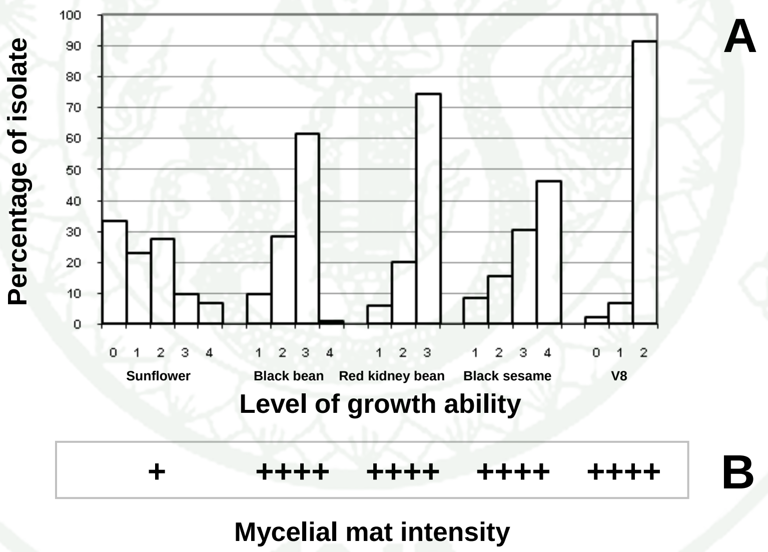

Figure 3 Growth ability of 132 *Phytophthora infestans* isolates on five selected media after seven days incubation. Level of growing ability of each isolate is rated using a number scale; 0 = no growth up to 4 = abundant (A). Mean comparison of mycelial intensity obtained from overall isolates on each medium (B). Where; + = sparse, ++++ = abundant.

Table 11 Recovery (%) and sporulation of *Phytophthora infestans* stored in media for 8 months

Medium[a]	Recovery of fungi (%)		No. of sporangia/cm^2 of medium surface[b]					
			4°C			18°C		
	4°C	18°C	1 mo.	5 mo.	8 mo.	1 mo.	5 mo.	8 mo.
BSS	100	100	4.2×10^4 b	1.1×10^5 bc	7.3×10^4 ns	4.9×10^4 cd	6.4×10^4 b	4.8×10^4 ns
RKB	100	100	1.3×10^5 a	7.5×10^4 c	4.8×10^4 ns	1.4×10^4 d	7.4×10^4 ab	4.8×10^4 ns
SFW	100	100	7.7×10^4 b	1.3×10^5 b	5.9×10^4 ns	9.4×10^4 bc	1.1×10^5 a	7.6×10^4 ns
RA	100	100	4.9×10^4 b	1.9×10^5 a	4.5×10^4 ns	1.7×10^5 a	9.1×10^4 ab	6.8×10^4 ns
BB	100	100	4.4×10^4 b	1.8×10^5 a	5.0×10^4 ns	1.0×10^5 b	1.1×10^5 a	5.4×10^4 ns

[a]BSS = black sesame agar, RKB = red kidney bean agar, SFW = sunflower agar, RA = rye A agar and BB = black bean agar were supplemented with 0.05 g of *β*-sitosterol

[b]Mean no. of sporangia. Values followed by the same letter for each column are not significantly different at $P = 0.05$ according to Duncan's new multiple range test. ns = non significant

Chapter 2

Morphological characteristics of *Phytophthora infestans*

1. Micro-morphology

1.1 Reproductive Propagules

The culture of *P. infestans* on rye A agar at 18^{o}C in dark condition showed white-concentric ring to cottony colony. The culture on rye A agar showed only cottony colony. A mycelium was hyaline, branched, and coenocytic. Sporangia formed on the branch of sporangiophore which had swelling at sites of sporangial formation. Sporangia were ovoid, ellipsoid to moniliform, tapering at the base and semipapillate (Fig. 4 and 5).

Length and breadth of sporangia ranged from 17.33-33.33 μm to 32.00-59.83 μm in diameter. Average size of L:B ratio was 1.8. Sizes of pedicel ranged from 0.81 to 3.91 μm. Average size of pedicel was 1.79 μm. *P. infestans* is heterothallic. Oospores were formed by antheridia and oogonia. Sizes of oospore formed in rye B agar were 28.58 to 36.00 μm in diameter (Table 12).

1.2 Variation of Reproductive Propagules based on Size

A group of these reproductive propagules of 132 isolates was enumerated after measuring on sizes of sporangia (L:B ratio), pedicel and oospore. Each reproductive propagule was divided into 3 groups (Fig. 6) as follows;

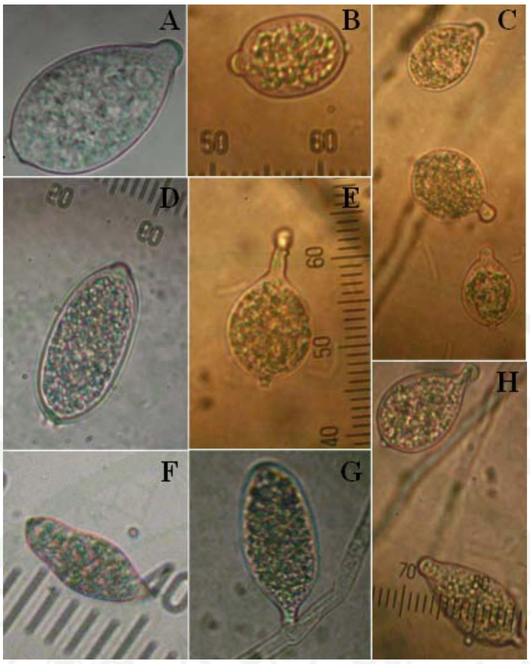

Figure 4 Range of sporangial shapes observed form 132 isolates of *Phytophthora infestans*. A and H, limoniform sporangium. B, D and G, ellipsoid sporangium. C, ovoid sporangium. E, direct germination of a globose sporangium. F, distorted sporangium.

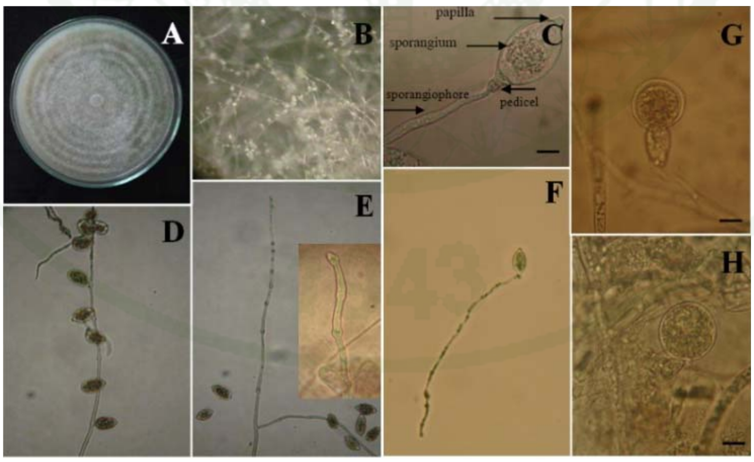

Figure 5 Macro- and micro- morphology of *Phytophthora infestans*. A, culture on rye A agar. B, sporangiophore with sporangia growing on potato slice. C, sporangium. D and E, sporangiophore. F, direct germination of sporangium. G and H, oospore. Scale bars = 10 μm

Table 12 Sizes of reproductive organs of *Phytophthora infestans* after 14 days incubation on rye A agar at 18°C in the dark condition

Isolate	Reproductive organ (μm)				
	Sporangium[a]		L:B[a]	Pedicel[b]	Oospore[c]
	Length	Breadth			
1	45.33	24.17	1.88	2.08	32.17
2	46.83	21.50	2.18	2.53	31.42
3	40.20	22.60	1.78	2.26	30.33
4	39.33	29.33	1.34	2.46	30.50
5	45.33	33.33	1.36	2.56	30.50
6	35.50	20.17	1.76	1.62	31.83
7	42.50	22.17	1.92	1.88	35.08
8	36.83	20.17	1.83	2.00	35.08
9	35.83	19.83	1.81	2.13	33.50
10	37.50	19.50	1.92	2.25	30.00
11	38.50	20.00	1.93	1.17	29.67
13	39.50	21.50	1.84	1.73	31.50
14	38.33	19.50	1.97	1.57	34.08
15	32.83	21.67	1.52	1.10	30.17
16	41.33	20.33	2.03	1.95	31.75
17	37.33	19.83	1.88	1.56	36.00
18	47.50	22.17	2.14	2.23	36.00
19	43.00	22.33	1.93	2.23	31.50
20	47.17	22.33	2.11	2.56	30.50
21	38.33	20.67	1.85	1.49	31.50
22	40.00	21.00	1.90	1.70	35.33
23	37.17	20.50	1.81	1.93	30.67
24	47.00	22.83	2.06	1.88	31.83
25	45.67	22.83	2.00	1.99	30.17
26	42.50	20.17	2.11	2.06	30.33
27	40.17	23.00	1.75	1.81	31.50
28	36.83	20.17	1.83	1.26	31.83
29	37.33	19.33	1.93	1.99	30.33
30	38.50	19.67	1.96	2.27	34.75
31	42.50	20.67	2.06	1.99	29.50
32	39.00	19.50	2.00	2.13	30.42
33	40.50	19.40	2.09	2.00	31.50
34	42.83	20.67	2.07	2.12	31.17
35	39.00	19.50	2.00	2.06	30.67
36	39.67	18.50	2.14	1.89	34.25
37	40.50	18.00	2.25	2.10	30.50
38	41.17	19.50	2.11	1.97	35.00
39	40.50	20.67	1.96	1.90	31.67
40	40.50	19.00	2.13	1.98	30.50
41	40.83	19.67	2.08	2.03	33.00

Table 12 (Continued)

Isolate	Reproductive organ (μm)				
	Sporangium[a]		L:B[a]	Pedicel[b]	Oospore[c]
	Length	Breadth			
42	40.00	19.17	2.09	2.16	32.83
43	40.67	20.67	1.97	1.57	31.00
44	47.83	22.67	2.11	2.17	30.67
45	42.33	20.83	2.03	1.84	33.00
46	40.00	20.83	1.92	1.63	32.83
47	41.50	20.17	2.06	1.70	34.83
48	46.00	20.67	2.23	2.46	33.67
49	34.50	18.33	1.88	1.41	32.67
50	46.33	22.50	2.06	2.18	35.08
51	38.67	21.17	1.83	1.58	30.33
52	44.17	20.17	2.19	2.04	30.83
54	40.67	21.17	1.92	1.70	33.67
56	42.33	17.33	2.44	1.07	34.17
57	44.67	22.67	1.97	1.46	31.33
58	39.00	20.33	1.92	1.51	30.00
59	39.50	19.33	2.04	2.12	30.83
60	45.00	22.50	2.00	1.84	31.17
62	36.67	20.50	1.79	0.92	30.00
63	39.17	21.67	1.81	1.66	32.33
64	41.50	21.67	1.92	0.98	31.00
65	45.67	25.83	1.77	1.75	31.00
66	45.83	25.50	1.80	1.25	30.83
67	42.67	21.17	2.02	3.12	28.58
68	34.17	19.50	1.75	1.40	31.33
69	39.67	20.00	1.98	2.03	32.17
70	45.17	21.67	2.08	2.03	31.58
72	37.33	20.00	1.87	1.79	34.17
73	37.50	20.83	1.80	1.57	30.00
74	41.17	22.50	1.83	1.76	30.67
75	41.17	21.67	1.90	2.18	30.58
76	43.00	24.83	1.73	2.04	30.33
77	39.33	19.33	2.03	1.45	30.00
78	37.33	18.67	2.00	1.62	30.17
79	32.00	20.00	1.60	2.28	30.42
80	41.33	20.17	2.05	1.52	31.33
81	46.17	20.33	2.27	2.00	31.50
82	43.83	22.33	1.96	1.97	34.17
83	44.33	26.17	1.69	2.18	31.50
84	41.17	26.83	1.53	1.38	30.08
85	45.67	27.50	1.66	1.95	35.00
86	44.33	28.67	1.55	1.51	31.00

Table 12 (Continued)

Isolate	Reproductive organ (μm)				
	Sporangium[a]		L:B[a]	Pedicel[b]	Oospore[c]
	Length	Breadth			
87	39.50	20.67	1.91	1.63	30.33
88	39.17	22.83	1.72	1.97	33.17
89	44.67	28.67	1.56	1.64	35.67
91	44.67	26.50	1.69	1.73	31.17
92	41.17	26.17	1.57	2.16	32.33
93	45.67	28.17	1.62	1.34	31.17
94	46.33	29.00	1.60	2.28	30.08
95	42.83	21.67	1.98	1.94	30.33
96	41.00	24.00	1.71	1.98	30.17
97	37.00	21.83	1.69	1.55	32.50
98	40.17	22.83	1.76	1.60	31.50
99	42.00	24.67	1.70	2.27	32.00
100	38.17	21.33	1.79	1.91	28.83
101	36.67	20.67	1.77	1.25	30.83
102	40.50	22.94	1.77	1.76	33.33
103	39.50	20.33	1.94	1.32	30.67
104	41.50	21.17	1.96	1.86	31.42
105	46.17	26.33	1.75	2.40	30.00
106	40.50	22.00	1.84	1.95	30.33
107	44.00	26.33	1.67	1.82	30.50
108	37.67	23.00	1.64	1.67	32.50
109	40.17	21.67	1.85	1.43	32.17
110	39.33	23.67	1.66	1.63	30.67
111	42.00	25.67	1.64	1.11	30.50
112	44.00	26.67	1.65	1.73	33.67
113	37.67	23.50	1.60	1.02	30.67
114	37.67	26.83	1.40	3.16	32.33
115	36.67	21.00	1.75	1.28	30.33
116	59.83	26.33	2.27	2.46	29.00
117	35.50	20.67	1.72	1.40	32.92
118	37.50	20.50	1.83	1.83	32.83
119	41.83	26.00	1.61	0.84	33.42
120	36.00	21.50	1.67	1.13	30.00
121	36.67	21.33	1.72	0.84	30.33
122	35.50	21.50	1.65	0.81	30.33
123	43.00	24.33	1.77	1.91	31.67
125	33.50	21.67	1.55	0.93	30.00
126	40.50	27.67	1.46	2.53	30.83
127	43.00	25.17	1.71	0.89	30.17
128	40.00	26.33	1.52	1.53	33.33
129	43.17	27.50	1.57	3.46	30.17

Table 12 (Continued)

Isolate	Reproductive organ (µm)				
	Sporangium[a]		L:B[a]	Pedicel[b]	Oospore[c]
	Length	Breadth			
131	45.33	27.67	1.64	3.91	30.58
132	38.17	23.83	1.60	1.41	30.50
133	34.67	22.00	1.58	1.43	30.50
134	47.50	25.17	1.89	2.37	30.83
135	36.17	21.67	1.67	1.22	30.00
136	34.17	20.33	1.68	1.12	30.50
137	34.83	19.83	1.76	1.08	31.50
138	33.50	22.67	1.48	1.07	31.17
139	35.17	19.67	1.79	1.08	29.42
140	34.50	20.17	1.71	1.06	30.00
Average	40.66	22.27	1.84	1.79	31.56
Standard Error	nd[d]	nd	0.087	0.187	0.750
LSD $_{(P=0.05)}$	nd	nd	0.243	0.520	2.085

[a]Sporangia were obtained from culture on rye A agar and determined at 100X magnification. Thirty sporangia of each isolate were calculated for mean. Least significant difference (LSD) test at *P* = 0.05 was used for a significant difference between isolates.

[b]Sporangia were obtained from culture on rye A agar and determined at 400X magnification. Thirty sporangia of each isolate were calculated for mean. Least significant difference (LSD) test at *P* = 0.05 was used for a significant difference between isolates.

[c]Oospores were obtained from culture on rye B agar and determined at 100X magnification. Thirty oospores of each isolate were calculated for mean. Least significant difference (LSD) test at *P* = 0.05 was used for a significant difference between isolates.

[d]nd = not determine

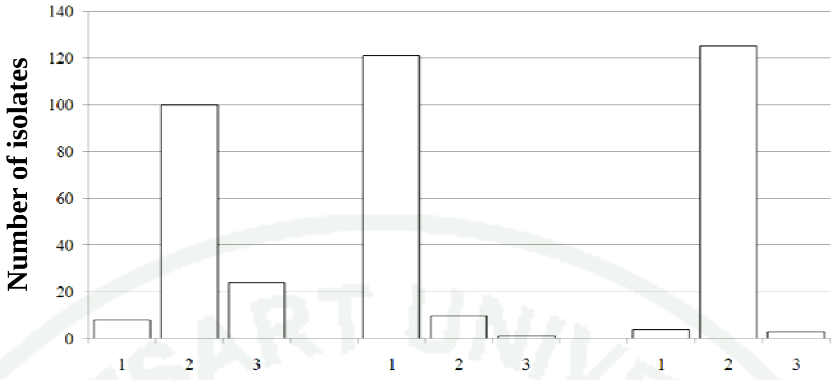

Figure 6 Group of 132 isolates of *Phytophthora infestans* divided by size of reproductive propagule. Histogram bar of each sporangial (L:B ratio) group represents 1 = 1.00-1.50, 2 = 1.60-2.00, and 3 = 2.10-2.50. Histogram bar of each pedicel group represents 1 = 0.50-2.00, 2 = 2.10-3.50, and 3 = 3.60-5.00 μm. Histogram bar of each oospore group represents 1 = 25.00-29.00, 2 = 30.00-35.00, and 3 = 36.00-40.00 μm.

1.2.1 L:B Ratio

The minimum and maximum of L:B ratio were 1.34 (isolate 4 and 93) and 2.44 (isolate 56), respectively. One hundred percentage of all isolates had L:B ratio ranging from 1.6-2.0 (group 2). Group 2 had the highest number of the isolates and higher than group 1 and 3 about 12- and 4-fold, respectively (Fig. 6).

1.2.2 Pedicel Length

The shortest and longest of pedicel were 0.81 (isolate 122) and 3.91 (isolate 131) μm, respectively. One hundred twenty-one percentage of all isolates had pedicel sizes ranging from 0.50-2.00 (group 1). Group 1 had the highest number of the isolates and higher than group 2 and 3 about 12- and 120-fold, respectively (Fig. 6).

1.2.3 Oospore Diameter

The smallest and biggest of oospore were 28.58 and 36.00 μm in diameter, respectively, that containing 1 and 2 isolate (s), respectively. One hundred twenty-five percentage of all isolates had oospore sizes ranging from 30.00-35.00 (group 2). Group 2 had the highest number of the isolates and higher than group 1 and 3 about 31- and 42-fold, respectively (Fig. 6).

1.3 Relations between Size of Reproductive Propagules and Source of Isolate

No isolate from Nong Han District was recorded in the group of biggest size (group 3) for L:B ratio, pedicel and oospore (Table 13). The relations between reproductive propagules and source of isolate were analyzed by Chi-squared test at $P = 0.01$. Pearson Chi-square and Asymp. Sig. (2-tailed) values of L:B ratio were 13.458 and 0.337 showing that size of sporangia had no relation with source of isolate (Appendix Table E2). Pearson Chi-square and Asymp. Sig. (2-tailed) values of pedicel were 5.697 and 0.931 showing that size of pedicel had no relation with source of isolate (Appendix Table E3). Pearson Chi-square and Asymp. Sig. (2-tailed) values of oospore were 16.827 and 0.156 showing that size of oospore had no relation with source of isolate (Appendix Table E4).

1.4 Clusters of *Phytophthora infestans* Isolates Using Micro-morphologies

Twelve clusters of *P. infestans* Thai isolates were classified by using all 3 micro-morphologies (Table 14). The cluster 4 contained most number of isolates (70.5% of Thai isolates) while the 5 clusters (cluster 6, 7, 8, 10 and 11) contained least number of isolates (0.8% of Thai isolates for each cluster). Shannon and Evenness indices of the clusters of micro-morphology were 1.136 and 0.457, respectively.

Table 13 Number of *Phytophthora infestans* isolates collected in different regions of northern Thailand and classified into each group of micro-morphology

Province[a]	District	Sub-District	Number of isolate[b]								
			Group based on L:B ratio[c]			Group based on pedicel[d]			Group base on oospore[e]		
			1	2	3	1	2	3	1	2	3
CM	Fa	-	0	4	0	4	0	0	0	4	0
	SS	-	0	3	0	3	0	0	0	3	0
	SS	CMK	4	24	9	33	4	0	1	35	1
	SS	MFK	1	17	5	21	1	1	0	23	0
	SS	NH	0	38	9	45	2	0	1	45	1
	Ph	LK	1	4	0	5	0	0	1	4	0
TK	PP	RTP	2	10	1	10	3	0	1	11	1
		Total	8	100	24	121	10	1	4	125	3

[a] Isolate sources : Province; CM = Chiang Mai, T = Tak
: District; Fa = Fang, SS = San Sai, Ph = Phrao, PP = Phop Phra
: Sub-District; NH = Nong Han, MFK = Mae Faek Mai, CMK = Chedi Mae Krua, LK = Long Khot, RTP = Ruam Thai Phatthana
: - = absent

[b]Chi-square was used to analysis the relation in the sizes of reproductive propagules and the source of isolate.

[c]Group of sporangia (L:B ratio); 1 = 1.00-1.50, 2 = 1.60-2.00, and 3 = 2.10-2.50.

[d]Group of pedicel; 1 = 0.50-2.00, 2 = 2.10-3.50, and 3 = 3.60-5.00 μm.

[e]Group of oospore; 1 = 25.00-29.00, 2 = 30.00-35.00, and 3 = 36.00-40.00 μm.

Table 14 Clusters of *Phytophthora infestans* Thai isolates classified by multi-characteristic of micro-morphology

	Group based on			Isolate		
cluster	L:B ratio[a]	Pedicel[b]	Oospore[c]	Number	Percentage	Name
1	1	1	2	4	3.0	15, 84, 128, 138
2	1	2	2	4	3.0	4, 5, 114, 126
3	2	1	1	2	1.5	100, 139
4	2	1	2	93	70.5	1, 3, 6, 7, 8, 9, 10, 11, 13, 14, 16, 19, 21, 22, 23, 25, 27, 28, 29, 30,32, 35, 39, 43, 45, 46, 49, 51, 54, 57, 58, 59, 60, 62, 63, 64, 65, 66, 68, 69, 72, 73, 74, 75, 76, 77, 78, 79, 82, 83, 85, 86, 87, 88, 91, 92, 93, 94, 95, 96, 97, 98, 99, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 115, 117, 118, 119, 120, 121, 122, 123, 125, 127, 132, 133, 134, 135, 136, 137, 140
5	2	1	3	2	1.5	17, 89
6	2	2	1	1	0.8	67
7	2	2	2	1	0.8	129
8	2	3	2	1	0.8	131
9	3	1	2	19	14.4	24, 26, 31, 33, 34, 36, 37, 38, 40, 41, 42, 44, 47, 50, 52, 56, 70, 80, 81
10	3	1	3	1	0.8	18
11	3	2	1	1	0.8	116
12	3	2	2	3	2.3	2, 20, 48
			Total	132	100	

[a]Group of sporangia (L:B ratio); 1 = 1.00-1.50, 2 = 1.60-2.00, and 3 = 2.10-2.50.

[b]Group of pedicel; 1 = 0.50-2.00, 2 = 2.10-3.50, and 3 = 3.60-5.00 μm.

[c]Group of oospore; 1 = 25.00-29.00, 2 = 30.00-35.00, and 3 = 36.00-40.00 μm.

2. Macro-morphology

2.1 Culture of *Phytophthora infestans* Thai isolates on 20% Clarified V8 Agar

When the fungus was cultured on V8 agar, growth ability and mycelial mat density were observed using following criteria; The growth abilities were divided into 3 levels (Fig. 7); level 0) colony diameter was less than 1.5 cm or only growth on the original mycelial plug, level 1) colony diameter was equal or more than 1.5 cm but less than 2.5 cm, and level 2) colony diameter was equal or more than 2.5 cm. The mycelial mat density were divided into 4 levels (Fig. 8); level 0) sparse, level 2) semi-moderate, level 3) moderate, and level 4) abundant.

Three (approximately 2%), 9 (approximately 7%) and 120 (approximately 91%) of Thai isolates were classified for level 0, 1 and 2, respectively, of the growth abilities. Three (approximately 2%), 6 (approximately 5%), 103 (approximately 78%), and 20 (approximately 15%), isolates were classified for level 0, 1, 2, 3, and 4, respectively, of the mycelial mat intensity.

On V8 agar, 129 (approximately 98%) and 3 of Thai and US isolates, respectively, grew well (Table 15). Three (approximately 2%) of Thai isolates which were isolate 14, 43 and 98 grew very poor (Table 15) and the mycelial mat intensity of colonies were very sparse. Isolate 14, 43 and 98 showed level 0 on both of the growth abilities and the mycelial mat densities. Colony of those isolates did not grow on the surface of V8 agar and were decided to unable to culture on this medium.

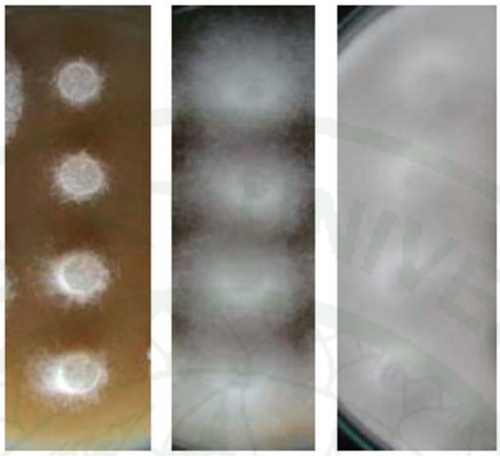

Figure 7 The levels of growth abilities of *Phytophthora infestans* on 20% unclarified V8 agar after 10 days incubation at 18^{o}C.

Figure 8 The levels of mycelial mat densities of *Phytophthora infestans* on 20% unclarified V8 agar after 10 days incubation at 18^{o}C.

2.2 Colonial characteristics of *Phytophthora infestans*

2.2.1 Rye A Agar

Three types; cottony (C), powdery (P) and concentric ring (CR) type, of colonies of *P. infestans* cultures were characterized on rye A agar at 14 days after incubation (Table 15 and Fig. 9). Thirty four isolates (26%) were cottony. Sixty nine isolates (52%) were powdery type. Twenty nine isolates (22%) were concentric ring type.

2.2.2 Potato Tuber Slice

Two types; mycelial (M) and sporangial (S) type, of colonies of *P. infestans* cultures were characterized on potato tuber slices at 7 days after incubation (Table 15 and Fig. 10). Eighty five isolates (64%) were mycelial type. Forty seven (36%) were sporangial type.

2.3 Cluster of *Phytophthora infestans* Isolates using Macro-morphologies

Twenty six clusters of *P. infestans* Thai isolates were classified by using macro-morphologies (Table 16). The cluster 20 contained most number of isolates (25.0% of Thai isolates) while the cluster 1, 2, 6, 7, 9, 10, 11, 12, 15, 17 ,19 and 23 contained least number of isolates (0.8% of Thai isolates for each cluster). Shannon and Evenness indices of the clusters of macro-morphology were 2.562 and 0.775, respectively.

Table 15 Cultural characteristics of *Phytophthora infestans* Thai isolates on rye A agar and 20% unclarified agar after 14 days incubation and potato tuber slice after 7 days incubation at 18°C

Isolate	Colony type[a]		V8 agar	
	Rye A agar	Potato tuber slice	Growth[b]	Density[c]
1	C	M	1	2
2	C	M	1	2
3	C	S	1	2
4	P	M	2	2
5	P	S	2	1
6	C	M	2	2
7	P	M	2	2
8	CR	S	2	2
9	C	M	2	3
10	C	M	2	3
11	C	M	2	2
13	C	S	2	2
14	P	M	0	0
15	CR	S	1	2
16	P	S	2	3
17	P	S	2	2
18	C	M	2	3
19	C	M	2	2
20	P	S	1	2
21	P	M	2	2
22	CR	M	1	2
23	P	S	2	2
24	C	S	2	1
25	C	M	2	2
26	C	M	2	2
27	P	M	2	3
28	C	S	2	3
29	P	M	2	2
30	P	S	2	2
31	P	M	2	3
32	CR	M	2	2
33	CR	S	2	2
34	P	M	2	1
35	CR	M	2	3
36	P	M	2	2

Table 15 (Continued)

Isolate	Colony type[a]		V8 agar	
	Rye A agar	Potato tuber slice	Growth[b]	Density[c]
37	CR	M	2	3
38	P	S	2	2
39	CR	M	2	2
40	P	S	2	2
41	C	M	2	2
42	P	M	2	2
43	P	M	0	1
44	P	M	2	2
45	C	M	2	2
46	P	S	2	1
47	P	S	2	2
48	P	M	2	2
49	P	M	2	2
50	CR	M	2	2
51	CR	M	2	2
52	CR	M	2	2
54	P	M	2	2
56	CR	M	2	2
57	P	M	2	2
58	CR	S	2	3
59	P	M	2	3
60	C	M	2	3
62	P	M	2	3
63	P	M	2	2
64	C	S	2	2
65	CR	M	2	2
66	CR	M	2	2
67	CR	S	2	2
68	P	M	2	2
69	CR	M	2	1
70	P	S	2	2
72	CR	M	2	2
73	P	M	2	2
74	P	M	2	2
75	P	M	2	3
76	P	M	2	2
77	C	M	2	3
78	P	S	2	2
79	P	M	2	2

Table 15 (Continued)

Isolate	Colony type[a]		V8 agar	
	Rye A agar	Potato tuber slice	Growth[b]	Density[c]
80	P	S	2	2
81	C	S	2	2
82	P	M	2	2
83	P	S	2	2
84	CR	M	2	2
85	CR	M	2	2
86	P	M	2	2
87	P	S	2	2
88	P	M	2	2
89	C	M	2	2
91	P	S	2	2
92	C	M	2	2
93	C	S	2	2
94	P	S	2	2
95	P	S	2	2
96	P	M	2	2
97	P	M	2	2
98	C	M	0	0
99	CR	M	2	2
100	P	S	2	2
101	P	M	2	2
102	C	M	2	3
103	C	M	2	2
104	C	M	1	1
105	C	S	2	2
106	C	M	2	2
107	P	S	2	2
108	CR	S	2	2
109	P	S	2	2
110	P	S	2	2
111	CR	M	2	2
112	C	M	2	3
113	P	M	2	2
114	CR	S	2	2
115	C	M	2	2
116	P	M	2	2
117	P	M	2	2
118	C	M	2	2

Table 15 (Continued)

Isolate	Colony type[a]		V8 agar	
	Rye A agar	Potato tuber slice	Growth[b]	Density[c]
119	P	S	2	3
120	P	M	2	2
121	CR	S	2	2
122	P	M	2	2
123	CR	M	2	3
125	CR	S	2	2
126	P	M	2	2
127	CR	S	2	2
128	P	S	2	2
129	C	M	2	2
131	CR	S	2	2
132	P	M	2	2
133	P	S	1	3
134	P	S	2	2
135	C	M	2	2
136	P	S	2	2
137	P	M	2	2
138	P	M	2	2
139	P	S	2	2
140	P	M	2	2
US-1	nd	nd	2	2
US-7	nd	nd	2	2
US-11	nd	nd	2	2

[a]Colonial type on rye A agar: cottony (C), powdery (P) and concentric ring (CR) type. Colonial type on infected potato tuber slice: Sporangial (S) type colony and mycelial (M) type colony. nd = not determined.

[b]Three levels of growth abilities on V8 agar; level 0) colony diameter was less than 1.5 cm or only growth on the original mycelial plug, level 1) colony diameter was equal or more than 1.5 cm but less than 2.5 cm, and level 2) colony diameter was equal or more than 2.5 cm.

[c]Three levels of mycelial mat densities on V8 agar; level 0) sparse, level 2) semi-moderate, level 3) moderate, and level 4) abundant.

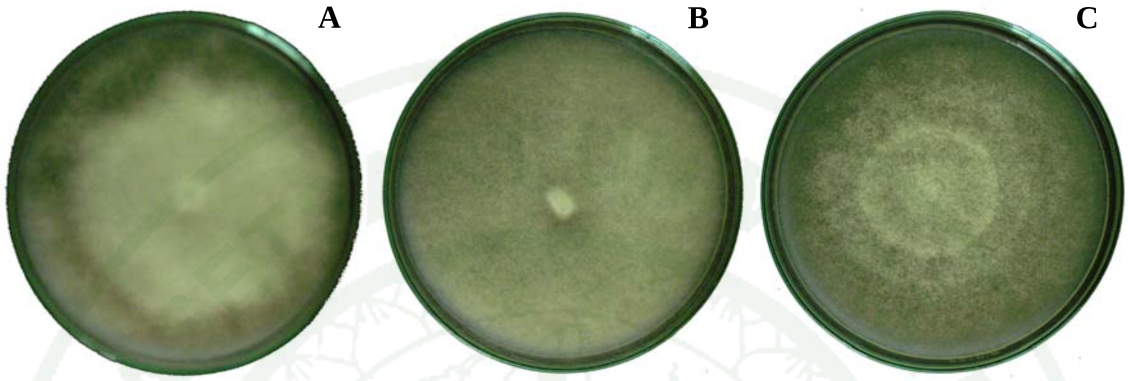

Figure 9 The colonial types of *Phytophthora infestans* cultures on rye A agar after 14 days incubation at 18°C. A, cottony type. B, powdery type. C, concentric ring type.

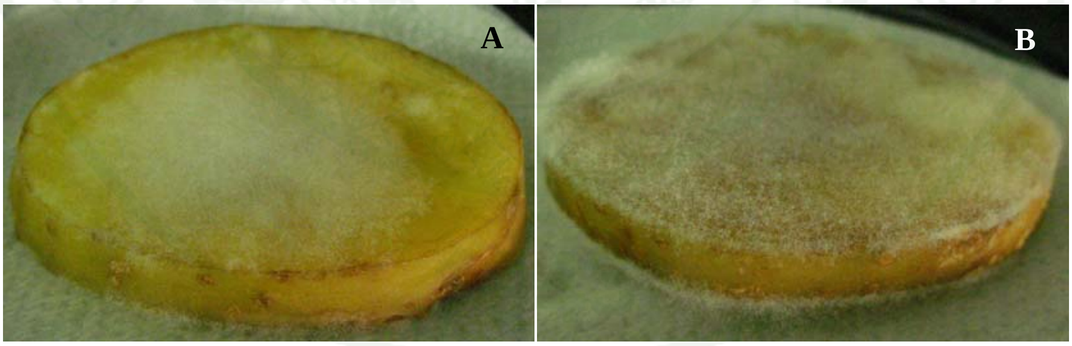

Figure 10 The colonial types of *Phytophthora infestans* cultures on potato tuber slices after 7 days incubation at 18°C. A, mycelial type. B, sporangial type.

Table 16 Clusters of *Phytophthora infestans* Thai isolates classified by multi-characteristics of macro-morphology

cluster	Colonial type: rye A agar[a]	Colonial type: Potato tuber slice[b]	V8 agar: Growth[c]	V8 agar: Density[d]	Isolate: Number	Isolate: Percentage	Isolate: Name
1	C	M	0	0	1	0.8	98
2	C	M	1	1	1	0.8	104
3	C	M	1	2	2	1.5	1, 2
4	C	M	2	2	15	11.4	6, 11, 19, 25, 26, 41, 45, 89, 92, 103, 106, 115, 118, 129, 135
5	C	M	2	3	7	5.3	9, 10, 18, 60, 77, 102, 112
6	C	S	1	2	1	0.8	3
7	C	S	2	1	1	0.8	24
8	C	S	2	2	5	3.8	13, 64, 81, 93, 105
9	C	S	2	3	1	0.8	28
10	CR	M	2	3	1	0.8	35
11	CR	M	1	2	1	0.8	22
12	CR	M	2	1	1	0.8	69
13	CR	M	2	2	13	9.8	32, 39, 50, 51, 52, 56, 65, 66, 72, 84, 85, 99, 111
14	CR	M	2	3,	2	1.5	37, 123
15	CR	S	1	2	1	0.8	15
16	CR	S	2	2	9	6.8	8, 33, 67, 108, 114, 121, 125, 127, 131
17	CR	S	2	3	1	0.8	58
18	P	M	0	0	2	1.5	14, 43
19	P	M	2	1	1	0.8	34
20	P	M	2	2	33	25.0	4, 7, 21, 29, 36, 42, 44, 48, 49, 54, 57, 63, 68, 73, 74, 76, 79, 82, 86, 88, 96, 97, 101, 113, 116, 117, 120, 122, 126, 132, 137, 138, 140
21	P	M	2	3	5	3.8	27, 31, 59, 62, 75
22	P	S	1	2	2	1.5	20, 70

Table 16 (Continued)

	Colonial type		V8 agar		Isolate		
cluster	rye A agar[a]	Potato tuber slice[b]	Growth[c]	Density[d]	Number	Percentage	Name
23	P	S	1	3	1	0.8	133
24	P	S	2	1	2	1.5	5, 46
25	P	S	2	2	21	15.9	17, 23, 30, 38, 40, 47, 78, 80, 83, 87, 91, 94, 95, 100, 107, 109, 110, 128, 134, 136, 139
26	P	S	2	3	2	1.5	16, 119
				Total	132	100	

[a]Colonial type on rye A agar: cottony (C), powdery (P) and concentric ring (CR) type.

[b]Colonial type on infected potato tuber slice: Sporangial (S) type colony and mycelial (M) type colony.

[c]Three levels of growth abilities on V8 agar; level 0) colony diameter was less than 1.5 cm or only growth on the original mycelial plug, level 1) colony diameter was equal or more than 1.5 cm but less than 2.5 cm, and level 2) colony diameter was equal or more than 2.5 cm.

[d]Three levels of mycelial mat densities on V8 agar; level 0) sparse, level 2) semi-moderate, level 3) moderate, and level 4) abundant.

Chapter 3

Pathogenicity and Aggressiveness of *Phytophthora infestans* Isolates

1. Pathogenicities of Thai Isolates on Primary and Alternative Host

1.1 Pathogenicity of *Phytophthora infestans* on Potato Leaflet, Potato Tuber Slice/Disc and Tomato Leaflet

Data on the fitness of the 132-Thai and 3-US isolates inoculated separately onto leaflets of potato and tomato or potato tuber slices/discs are given in Table 17. A large variation in fitness parameters was found between isolates.

1.1.1 Infection frequency

a. Potato leaflets cv. Atlantic and Spunta: The range of the infection frequency on both potato leaflets cultivars was 0.17 to 1 (Table 17). Ninety nine (75%) and 60 (45%) of 132 Thai isolates had the highest (1.00) frequency of infection on cv. Atlantic and Spunta, respectively.

b. Potato tuber discs cv. Atlantic and Spunta: All 132 (100%) of Thai isolates had the highest (1.00) frequency of infection on both cv. Atlantic and Spunta (Table 17).

c. Tomato leaflets cv. Delta and Seeda: The range of the infection frequency on tomato leaflets cv. Delta was 0.17 to 1.00 and cv. Seeda was 0.33 to 1.00 (Table 17). One hundred twenty two (94%) and 103 (79%) of 130 Thai isolates had the highest (1.00) frequency of infection on cv. Delta and Seeda, respectively.

The isolates showing the highest infection frequency on all host revealed the 34 aggressive isolates; 5, 6, 8, 13, 15, 16, 24, 25, 36, 37, 38, 39, 42, 46, 47, 51, 54, 56, 59, 62, 64, 77, 79, 80, 95, 99, 113, 114, 117, 121, 125, 133, 136 and 140, which associated in all 6 infected host.

1.1.2 Lesion area

a. Potato leaflets cv. Atlantic and Spunta: The range of the lesion area on potato leaflets cv. Atlantic was 21 to 100% and cv. Spunta was 13-100% (Table 17). Seventy four (56%) of 132 Thai isolates produced the large (92 to 100%) lesion areas on cv. Atlantic. Forty seven (36%) of 132 Thai isolates produced the large (90 to 100%) lesion areas on cv. Spunta.

b. Potato tuber slices cv. Atlantic and Spunta: The range of the lesion area on potato slices cv. Atlantic was 5 to 91% and cv. Spunta was 5 to 87% (Table 17). Eight (6%) of 131 Thai isolates produced the large (84 to 91%) lesion areas on cv. Atlantic. Three (2%) of 131 Thai isolates produced the large (79 to 87%) lesion areas on cv. Spunta.

c. Tomato leaflets cv. Delta and Seeda: The range of the lesion area on tomato leaflets cv. Delta was 3 to 84% and cv. Seeda was 2 to 67% (Table 17). Eleven (8%) of 130 Thai isolates produced the large (74 to 84%) lesion areas on cv. Delta. Three (2%) of 130 Thai isolates produced the large (59 to 67%) lesion areas on cv. Seeda.

The isolates producing the large lesion on all host revealed the aggressive isolate; 51, which associated with 4 infected host; potato leaflet cv. Atlantic and Spunta, potato tuber slice cv. Spunta and tomato leaflet cv. Delta.

1.1.3 Spore capacity

a. Potato leaflets cv. Atlantic and Spunta: The range of the spore capacity on potato leaflets cv. Atlantic was 22 to 2,733 spore/cm^2 lesion and cv. Spunta was 33 to 2,056 spore/cm^2 of lesion (Table 17). One (0.76%) of 132 Thai isolates, isolate 122, produced the highest number of sporangia on cv. Atlantic. One (0.76%) of 132 Thai isolates, isolate 40, produced the highest number of sporangia on cv. Spunta.

b. Potato tuber discs cv. Atlantic and Spunta: The range of the spore capacity on potato discs cv. Atlantic was 222-11,111 spore/ plug of tuber disc and cv. Spunta was 111-6,815 spore/ plug of tuber disc (Table 17). One (0.76%) of 130 Thai isolates, isolate 52, produced the highest number of sporangia on cv. Atlantic. Two (1.5%) of 130 Thai isolates, isolate 52 and 127, produced the high (6,407 to 6,815 spore/plug of tuber disc) number of sporangia on cv. Spunta.

c. Tomato leaflets cv. Delta and Seeda: The range of the spore capacity on tomato leaflets cv. Delta was 180 to 2,178 spore/cm^2 lesion and cv. Seeda was 148 to 2,100 spore/cm^2 of lesion (Table 17). Three (2%) of 130 Thai isolates, isolate 91, 102 and 106, produced the high (1,778 to 2,178 spore/cm^2 of lesion) number of sporangia on cv. Atlantic. One (1%) of 130 Thai isolates, isolate 106, produced the highest number of sporangia on cv. Seeda.

The isolates producing the high number of sporangia on all host revealed the 2 aggressive isolates; 52 and 106, which associated with 2 infected host differently; potato tuber discs cv. Atlantic and Spunta for isolate 52 and tomato leaflets cv. Delta and Seeda for isolate 106.

1.2 Pathogenicity of *Phytophthora infestans* on potato leaflets, potato slices/discs and tomato leaflets

Mean of value of fitness parameters obtained from Thai isolates were used for comparison between infected hosts (Table 18). Potato leaflets cv. Spunta (0.82) and tomato leaflets cv. Seeda (0.93) had significantly lower frequency of infection than another hosts whereas potato tuber discs cv. Atlantic and Spunta and tomato leaflet cv. Delta had highest frequency of infection (0.98-1.00). Tomato leaflets cv. Seeda (18%) had significantly smaller area of lesion than another hosts whereas potato leaflet cv. Atlantic (87%) had largest area of lesion. The isolates produced number of sporangia on both 2 cultivars of infected potato and tomato (292-635 spore/cm^2 of lesion) leaflets less than on infected potato discs (1,816-2,162 spore/cm^2 of lesion).

Table 17 Pathogenicity of *Phytophthora infestans* on potato leaflet, potato tuber slice/disc and tomato leaflet after 5 days inoculation

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
1	0.83	0.83	1.00	1.00	1.00	1.00	83	83	29	18	58	22	-	-	556	370	213	247
2	1.00	0.33	1.00	1.00	1.00	1.00	100	33	34	17	69	19	-	-	1,148	741	-	-
3	1.00	0.50	1.00	1.00	1.00	1.00	100	50	14	9	67	17	-	-	889	556	-	-
4	1.00	0.50	1.00	1.00	1.00	1.00	100	50	21	15	52	21	-	-	333	1,333	180	547
5	1.00	1.00	1.00	1.00	1.00	1.00	100	100	23	27	54	19	22	-	370	185	-	-
6	1.00	1.00	1.00	1.00	1.00	1.00	100	75	46	32	58	16	-	67	3037	370	-	-
7	1.00	0.50	1.00	1.00	1.00	0.83	90	52	32	28	20	12	133	-	1,111	926	-	-
8	1.00	1.00	1.00	1.00	1.00	1.00	100	68	31	20	45	17	78	533	1,556	1,296	-	-
9	1.00	0.67	1.00	1.00	1.00	1.00	100	67	42	33	24	18	-	-	3,778	2,556	271	453
10	0.83	0.83	1.00	1.00	1.00	1.00	83	90	36	66	14	8	167	-	407	296	582	476
11	1.00	0.83	1.00	1.00	1.00	1.00	100	69	32	32	60	13	44	-	2,630	1,148	275	648
13	1.00	1.00	1.00	1.00	1.00	1.00	100	100	32	44	59	22	-	-	2,185	407	-	-
14	0.67	0.83	1.00	1.00	1.00	1.00	83	83	27	20	18	10	33	-	1,593	5,481	-	-
15	1.00	1.00	1.00	1.00	1.00	1.00	100	61	10	18	53	29	56	-	1,407	1,111	285	528
16	1.00	1.00	1.00	1.00	1.00	1.00	100	100	22	44	50	23	33	-	2,630	1,037	-	-
17	1.00	1.00	1.00	1.00	1.00	0.83	100	70	32	30	48	8	789	-	1,889	1,889	579	392
18	1.00	0.83	1.00	1.00	1.00	1.00	100	83	24	31	69	34	-	-	1,444	1,074	-	-
19	1.00	0.83	1.00	1.00	1.00	0.83	100	82	46	41	69	12	-	-	2,889	2,222	813	490

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
20	0.83	0.67	1.00	1.00	1.00	1.00	100	60	14	11	78	18	22	-	370	148	-	-
21	1.00	1.00	1.00	1.00	nd	nd	100	74	44	47	nd	nd	1,267	-	-	-	nd	nd
22	1.00	0.83	1.00	1.00	1.00	1.00	100	79	41	31	34	17	-	-	1,370	1,296	-	-
23	0.50	0.83	1.00	1.00	1.00	1.00	67	83	25	11	56	24	-	-	1,519	1,407	-	-
24	1.00	1.00	1.00	1.00	1.00	1.00	100	100	35	23	67	13	-	-	4,074	741	-	-
25	1.00	1.00	1.00	1.00	1.00	1.00	100	100	55	36	62	25	56	-	3,778	3,704	-	-
26	1.00	0.67	1.00	1.00	1.00	1.00	100	52	21	40	62	17	-	-	1,667	1,556	232	412
27	1.00	0.67	1.00	1.00	1.00	1.00	71	67	51	51	62	12	-	-	1,926	1,481	-	-
28	1.00	0.33	1.00	1.00	1.00	1.00	100	46	37	38	26	11	44	-	1,444	1,074	-	-
29	0.83	0.67	1.00	1.00	1.00	1.00	83	48	28	33	62	19	-	-	5,556	4,444	-	-
30	1.00	0.83	1.00	1.00	1.00	1.00	100	83	36	61	15	20	-	-	1,815	1,556	-	-
31	1.00	0.33	1.00	1.00	1.00	0.83	100	33	64	43	53	16	89	-	704	407	-	-
32	0.83	1.00	1.00	1.00	1.00	1.00	83	100	12	36	62	17	-	-	333	296	249	264
33	1.00	0.50	1.00	1.00	1.00	1.00	100	45	70	63	72	18	-	-	4,074	5,704	-	-
34	1.00	0.83	1.00	1.00	1.00	1.00	100	67	76	60	69	32	-	-	1,741	1,444	-	-
35	0.83	1.00	1.00	1.00	1.00	1.00	67	100	64	32	71	52	78	-	3,222	2,037	-	-
36	1.00	1.00	1.00	1.00	1.00	1.00	100	96	31	43	27	67	-	-	3,407	2,296	-	-
37	1.00	1.00	1.00	1.00	1.00	1.00	91	97	32	41	77	25	33	-	2,037	1,667	-	-

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
38	1.00	1.00	1.00	1.00	1.00	1.00	100	92	37	25	74	26	-	-	2,815	1,926	-	-
39	1.00	1.00	1.00	1.00	1.00	1.00	100	100	30	37	28	18	200	-	3,815	1,222	464	378
40	1.00	0.67	1.00	1.00	1.00	1.00	100	51	24	29	65	15	344	2,056	1,593	1,185	-	-
41	1.00	0.83	1.00	1.00	1.00	1.00	100	66	51	31	60	27	-	-	2,074	1,630	-	-
42	1.00	1.00	1.00	1.00	1.00	1.00	100	65	43	84	73	19	-	-	2,889	2,481	-	-
43	0.83	1.00	1.00	1.00	1.00	1.00	83	100	22	22	73	28	678	-	2,852	3,963	-	-
44	1.00	1.00	1.00	1.00	nd	nd	100	100	nd	nd	nd	nd	22	-	nd	nd	nd	nd
45	1.00	0.50	1.00	1.00	1.00	1.00	100	50	39	24	64	23	111	-	4,889	5,333	-	-
46	1.00	1.00	1.00	1.00	1.00	1.00	92	96	21	29	67	29	-	-	3,667	3,222	-	-
47	1.00	1.00	1.00	1.00	1.00	1.00	100	97	44	42	61	24	-	-	3,222	2,778	-	-
48	0.67	1.00	1.00	1.00	1.00	1.00	67	94	24	16	71	17	89	-	2,852	2,407	256	317
49	1.00	1.00	1.00	1.00	1.00	1.00	100	100	56	44	79	14	-	33	2,667	4,630	-	-
50	1.00	0.67	1.00	1.00	1.00	0.83	100	67	61	31	80	6	-	-	2,889	2,889	-	-
51	1.00	1.00	1.00	1.00	1.00	1.00	100	100	28	80	78	7	-	-	3,407	3,037	-	-
52	1.00	0.83	1.00	1.00	1.00	1.00	100	83	59	73	68	18	-	-	11,111	6,815	530	344
54	1.00	1.00	1.00	1.00	1.00	1.00	100	100	44	40	74	18	44	444	5,370	4,556	-	-
56	1.00	1.00	1.00	1.00	1.00	1.00	100	100	59	32	42	30	-	-	2,000	1,593	-	-
57	1.00	0.67	1.00	1.00	1.00	1.00	100	67	32	41	38	59	-	-	2,481	2,074	427	-

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
58	1.00	0.33	1.00	1.00	1.00	1.00	100	18	23	48	65	38	-	307	7,815	3,333	-	-
59	1.00	1.00	1.00	1.00	1.00	1.00	93	100	33	14	19	54	-	-	1,593	1,259	-	-
60	1.00	0.50	1.00	1.00	1.00	1.00	100	50	63	31	21	53	167	-	1,963	1,630	-	-
62	1.00	1.00	1.00	1.00	1.00	1.00	100	83	25	26	70	33	-	-	5,000	4,444	1,086	-
63	1.00	0.83	1.00	1.00	1.00	1.00	100	54	62	68	36	24	44	-	778	481	-	-
64	1.00	1.00	1.00	1.00	1.00	1.00	100	100	62	52	58	21	33	156	1,370	1,037	593	-
65	1.00	0.17	1.00	1.00	1.00	1.00	100	17	23	35	55	28	33	-	1,037	3,222	-	-
66	1.00	0.83	1.00	1.00	1.00	1.00	100	75	28	57	60	26	111	370	3,333	3,222	-	-
67	1.00	0.67	1.00	1.00	1.00	1.00	100	65	53	38	23	28	189	-	2,148	1,667	-	-
68	1.00	0.67	1.00	1.00	1.00	1.00	99	67	41	41	71	23	33	111	4,444	4,000	-	-
69	1.00	0.50	1.00	1.00	1.00	1.00	100	34	33	54	10	27	-	33	2,889	2,444	-	-
70	1.00	0.83	1.00	1.00	1.00	1.00	100	70	13	65	57	24	-	-	3,148	2,741	-	-
72	1.00	0.67	1.00	1.00	1.00	0.67	100	67	9	10	14	5	89	-	222	111	-	-
73	1.00	1.00	1.00	1.00	1.00	0.83	100	96	36	42	59	4	256	-	296	148	-	-
74	1.00	0.83	1.00	1.00	1.00	1.00	100	83	31	21	18	12	-	-	2,889	2,519	-	-
75	1.00	0.83	1.00	1.00	1.00	1.00	100	70	29	37	14	19	-	-	667	1,556	-	-
76	1.00	0.83	1.00	1.00	1.00	1.00	100	83	29	32	63	11	22	-	1,481	5,185	-	-
77	1.00	1.00	1.00	1.00	1.00	1.00	100	85	36	49	66	10	-	-	2,704	2,407	-	-

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
78	1.00	0.83	1.00	1.00	1.00	1.00	100	83	36	41	68	17	300	-	5,926	5,556	667	-
79	1.00	1.00	1.00	1.00	1.00	1.00	100	100	37	39	15	17	-	-	778	519	-	-
80	1.00	1.00	1.00	1.00	1.00	1.00	100	100	88	50	32	13	-	-	778	444	-	-
81	1.00	0.83	1.00	1.00	0.67	1.00	100	55	11	32	10	6	-	-	1,037	556	-	-
82	1.00	1.00	1.00	1.00	0.83	0.50	100	73	53	30	13	2	-	-	1,148	370	611	-
83	0.67	1.00	1.00	1.00	1.00	0.50	83	95	61	41	21	3	-	200	556	370	-	-
84	1.00	1.00	1.00	1.00	1.00	0.67	100	100	85	22	28	5	56	-	3,037	4,296	626	-
85	1.00	0.83	1.00	1.00	1.00	1.00	100	60	59	28	35	11	-	-	1,296	1,444	-	-
86	1.00	1.00	1.00	1.00	0.83	1.00	86	88	41	32	17	10	22	-	556	667	489	-
87	1.00	0.50	1.00	1.00	1.00	0.67	100	50	91	86	70	5	56	267	2,259	667	684	519
88	1.00	0.83	1.00	1.00	0.67	1.00	92	100	13	42	8	26	-	56	370	444	600	148
89	0.67	0.50	1.00	1.00	1.00	1.00	43	98	48	32	33	5	-	-	1,259	741	778	-
91	1.00	0.83	1.00	1.00	0.50	1.00	75	73	5	21	4	13	-	-	1,741	1,185	2,178	-
92	1.00	0.83	1.00	1.00	0.67	0.67	100	84	11	27	7	4	33	-	630	370	-	-
93	0.83	0.83	1.00	1.00	1.00	0.33	83	57	47	38	16	2	-	336	2,889	4,000	867	611
94	0.67	1.00	1.00	1.00	1.00	1.00	71	94	35	21	17	6	244	-	3,074	2,889	-	-
95	1.00	1.00	1.00	1.00	1.00	1.00	100	100	21	32	27	7	22	122	2,481	2,074	489	-
96	1.00	1.00	1.00	1.00	1.00	0.83	74	100	33	33	25	9	481	-	1,815	3,815	-	-

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
97	1.00	0.67	1.00	1.00	1.00	1.00	69	36	28	68	11	9	1,048	-	1,259	815	-	-
98	0.83	0.33	1.00	1.00	1.00	0.50	59	33	69	60	31	2	73	-	1,630	1,259	-	-
99	1.00	1.00	1.00	1.00	1.00	1.00	73	100	39	52	13	9	67	78	1,593	852	978	-
100	0.83	1.00	1.00	1.00	1.00	0.67	67	96	27	48	28	4	111	-	1,778	1,593	472	-
101	0.83	0.83	1.00	1.00	1.00	0.83	63	44	45	41	18	7	-	-	2,481	2,074	-	-
102	1.00	0.67	1.00	1.00	1.00	0.83	56	58	28	41	12	6	317	186	1,815	1,259	1,778	533
103	1.00	0.83	1.00	1.00	1.00	0.67	75	61	58	35	27	5	721	-	2,333	1,148	722	-
104	0.50	1.00	1.00	1.00	1.00	1.00	44	94	33	29	26	8	-	-	1,111	593	-	-
105	0.83	1.00	1.00	1.00	1.00	1.00	66	100	80	25	11	7	64	-	1,630	704	-	-
106	0.83	0.67	1.00	1.00	1.00	0.50	61	59	35	66	17	2	741	-	4,259	407	2,074	2,100
107	0.67	0.83	1.00	1.00	1.00	0.33	38	55	29	36	34	6	259	-	3,259	2,889	506	-
108	1.00	0.83	1.00	1.00	0.67	1.00	85	67	29	9	6	9	156	-	333	148	722	-
109	0.83	0.67	1.00	1.00	1.00	0.50	79	53	87	52	42	2	167	-	667	333	-	400
110	0.17	0.67	1.00	1.00	1.00	1.00	21	67	24	24	14	10	-	-	778	444	-	-
111	0.83	1.00	1.00	1.00	1.00	0.67	75	100	38	39	13	3	-	44	3,630	2,889	622	-
112	0.83	1.00	1.00	1.00	1.00	0.67	73	89	69	37	32	4	-	-	1,667	741	-	-
113	1.00	1.00	1.00	1.00	1.00	1.00	75	100	48	60	23	10	422	122	519	2,407	217	1,100
114	1.00	1.00	1.00	1.00	1.00	1.00	93	77	52	23	38	8	178	44	1,556	3,519	-	-

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
115	1.00	0.50	1.00	1.00	1.00	1.00	67	39	90	37	39	10	-	-	852	852	-	-
116	1.00	0.50	1.00	1.00	1.00	1.00	85	50	28	23	18	15	256	-	2,407	2,074	630	-
117	1.00	1.00	1.00	1.00	1.00	1.00	79	84	70	31	18	5	-	-	1,148	1,333	-	-
118	0.83	0.50	1.00	1.00	0.83	1.00	73	50	26	31	13	6	-	-	1,741	1,259	-	-
119	1.00	1.00	1.00	1.00	1.00	0.83	70	88	28	21	13	4	456	-	370	519	489	-
120	0.83	1.00	1.00	1.00	1.00	0.83	86	92	85	33	36	4	-	267	3,296	1,185	633	867
121	1.00	1.00	1.00	1.00	1.00	1.00	68	100	34	25	15	12	-	-	741	852	-	-
122	1.00	1.00	1.00	1.00	1.00	0.83	80	90	57	71	44	4	2,733	474	2,333	741	-	-
123	0.83	0.83	1.00	1.00	1.00	1.00	75	69	48	16	79	42	1,044	-	2,519	2,481	-	-
125	1.00	1.00	1.00	1.00	1.00	1.00	93	97	54	43	73	30	1,122	400	630	556	-	-
126	1.00	0.50	1.00	1.00	1.00	1.00	92	22	17	29	24	32	-	-	2,000	2,000	-	-
127	0.83	0.67	1.00	1.00	1.00	1.00	83	51	85	43	71	28	-	-	6,111	6,407	-	-
128	0.83	0.83	1.00	1.00	1.00	1.00	75	83	42	18	80	26	122	352	259	222	-	-
129	1.00	0.83	1.00	1.00	1.00	1.00	63	83	45	24	82	26	-	-	1,222	1,556	-	-
131	0.83	1.00	1.00	1.00	1.00	1.00	65	76	28	23	74	19	74	-	1,222	2,852	-	-
132	1.00	0.83	1.00	1.00	1.00	1.00	90	75	39	26	69	25	965	193	2,593	1,704	-	-
133	1.00	1.00	1.00	1.00	1.00	1.00	79	100	18	6	68	16	-	-	741	1,259	-	-
134	1.00	0.17	1.00	1.00	1.00	1.00	100	14	55	28	63	22	400	756	4,630	1,926	343	160

Table 17 (Continued)

Isolate[a]	Aggressiveness parameter[b]																	
	Infection frequency						Lesion area						Spore capacity					
	Potato leaflet		Potato disc		Tomato leaflet		Potato leaflet		Potato slice		Tomato leaflet		Potato leaflet		Potato disc		Tomato leaflet	
	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See	At	Sp	At	Sp	Del	See
135	0.50	1.00	1.00	1.00	1.00	1.00	32	100	89	59	84	23	-	33	2,370	630	-	-
136	1.00	1.00	1.00	1.00	1.00	1.00	76	63	74	35	76	9	78	-	1,778	1,185	-	-
137	1.00	0.83	1.00	1.00	1.00	1.00	97	100	51	17	65	12	2,056	-	407	333	196	-
138	1.00	0.83	1.00	1.00	1.00	1.00	73	83	52	35	51	22	1,632	-	2,000	1,148	-	-
139	0.67	1.00	1.00	1.00	1.00	1.00	55	100	65	48	50	28	974	-	1,407	704	-	570
140	1.00	1.00	1.00	1.00	1.00	1.00	66	86	40	60	68	66	1,290	122	1,037	444	-	-
US-1	0.83	1.00	1.00	1.00	1.00	0.67	76	100	37	7	80	6	122	-	370	111	-	-
US-7	1.00	0.67	1.00	1.00	0.83	0.33	70	67	9	21	14	2	256	-	407	185	-	-
US-11	0.83	0.83	1.00	1.00	1.00	0.33	75	91	12	15	26	3	-	-	370	148	-	-
Mean	**0.94**	**0.82**	**1.00**	**1.00**	**0.98**	**0.93**	**87**	**76**	**41**	**37**	**56**	**18**	**355**	**292**	**2,162**	**1,816**	**635**	**544**
SE	**0.10**	**0.16**	**0**	**0**	**0.04**	**0.09**	**10.15**	**17.20**	**5.93**	**4.83**	**4.72**	**3.76**	**66**	**160**	**313**	**299**	**128**	**154**
LSD	**0.26**	**0.45**	**ns**	**ns**	**0.11**	**0.24**	**28.13**	**47.67**	**16.46**	**13.39**	**13.08**	**10.43**	**183**	**451**	**867**	**829**	**359**	**436**

Table 17 (Continued)

[a]Least significant difference (LSD) test at *P* = 0.05 was used for a significant difference between isolates.

[b]Aggressiveness parameters: infection frequency (the proportion of inoculated leaflets or tuber discs infected), lesion area (percent of lesion) and sporulation capacity (spore/cm^2 of lesion), of *P*. *infestans* isolates which infected on potato leaflets and tuber slices cv. Atlantic (At) and Spunta (Sp) and tomato leaflets cv. Delta (Del) and Seeda (See) at 5 days after inoculation. nd = not determined.

"-" = the isolate produced a few sporangia on infected plant, uncountable using heamacytometer.

Table 18 Comparison on pathogenicity of *Phytophthora infestans* on potato leaflet, potato and tomato leaflet after 5 days inoculation

Host	Cultivar	Aggressiveness parameter[a]		
		Infection frequency[b]	Lesion area coverage [c]	Spore capacity[d]
Potato leaflet	Atlantic	0.94 b	87 a	355 b
	Spunta	0.82 c	76 b	292 b
Potato tuber	Atlantic	1.00 a	41 cd	2,162 a
	Spunta	1.00 a	37 d	1,816 a
Tomato leaflet	Delta	0.98 a	56 cd	635 b
	Seeda	0.93 c	18 e	544 b

[a]Within a row, figures followed by the same letter are not significantly different (*P*=0.05) according to

Least significant difference (LSD).

[b]Infection frequency: proportion of leaflets or tuber disc (0.7 cm diameter, 0.5 cm thick) inoculated which were infected.

[c]Lesion area coverage: area (%) of lesions produced per leaflet or tuber slice.

[d]Sporulation capacity: number of sporangia produced per square centimeter of lesion on leaflet or tuber disc (0.7 cm diameter, 0.5 cm thick).

1.3 Aggressiveness of *Phytophthora infestans* Thai isolates

P. infestans isolates were classified into a group of aggressiveness parameters such as infection frequency, lesion area, and spore capacity.

1.3.1 Infection frequency

a. Potato leaflets

1) cv. Atlantic: Value of infection frequency of the isolates infected on potato leaflets cv. Atlantic ranging from 0.17 to 1.00. The isolates were divided using infection frequency value to 3 groups; 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 =

0.71-1.00. Group 1 had the lowest values and composed of 1 isolate collected from Nong Han Sub-District whereas group 3 had the highest value and composed of 121 isolates (Table 19). Mean, median and mode of infection frequency value were 0.94, 1.00 and 1.00, respectively. The values had negative skewed distribution or curve skewed to the left that meant a most of isolates had high value of frequency infection.

2) cv. Spunta: Value of infection frequency of the isolates infected on potato leaflets cv. Spunta ranging from 0.17 to 1.00. The isolates were divided using infection frequency value to 3 groups; 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00. Group 1 had the lowest values and composed of 7 isolates collected from San Sai District whereas group 3 had the highest value and composed of 95 isolates (Table 19). Mean, median and mode of infection frequency value were 0.82, 0.83 and 1.00, respectively. The values had negative skewed distribution.

b. Potato tuber discs

All 132 Thai isolates had 100% frequency of infection (value = 1.00) on both cv. Atlantic and Spunta (Table 19). Mean, median and mode of infection frequency value were 1.00, 1.00 and 1.00, respectively. The values had normal or symmetry distribution that meant all of isolates had equal value of frequency infection.

c. Tomato leaflets

1) cv. Delta: Value of infection frequency of the isolates infected on tomato leaflets cv. Delta ranging from 0.50 to 1.00. The isolates were divided using infection frequency value to 3 groups; 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00. Group 1 had the lowest values and no isolate contained whereas group 3 had the highest value and composed of 125 isolates (Table 19). Mean, median and mode of infection frequency value were 0.98, 1.00 and 1.00, respectively. The values had negative skewed distribution.

2) cv. Seeda: Value of infection frequency of the isolates infected on tomato leaflets cv. Seeda ranging from 0.33-1.00. The isolates were divided using infection frequency value to 3 groups; 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00. Group 1 had the lowest values and composed of 2 isolates collected from Chedi Mae Krua and Mae Faek Mai Sub-Districts whereas group 3 had the highest value and composed of 115 isolates (Table 19). Mean, median and mode of infection frequency value were 0.93, 1.00 and 1.00, respectively. The values had negative skewed distribution.

1.3.2 Lesion area

a. Potato leaflets

1) cv. Atlantic: Area coverage (%) of lesion produced by the isolates on potato leaflet cv. Atlantic ranging from 21 to 100%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%. Group 1 had the smallest area and composed of 1 isolate collected from Nong Han Sub-District whereas group 4 had the largest area and composed of 97 isolates (Table 20). Mean, median and mode of lesion area value were 87.41, 100.00 and 100.00, respectively. The values had negative skewed distribution.

2) cv. Spunta: Area coverage (%) of lesion produced by the isolates on potato leaflet cv. Spunta ranging from 13 to 100%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%. Group 1 had the smallest area and composed of 4 isolates collected from Nong Han and Ruam Thai Phatthana Sub-Districts whereas group 4 had the largest area and composed of 72 isolates (Table 20). Mean, median and mode of lesion area value were 75.65, 83.00 and 100.00, respectively. The values had negative skewed distribution.

b. Potato tuber slices

1) cv. Atlantic: Area coverage (%) of lesion produced by the isolates on potato tuber slices cv. Atlantic ranging from 5 to 91%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%. Group 1 had the smallest area and composed of 27 isolates whereas group 4 had the largest area and composed of 10 isolates (Table 20). Mean, median and mode of lesion area value were 41.00, 36.00 and 27.75, respectively. The values had positive skewed distribution or curve skewed to the right that meant a most of isolates produced small area of lesion on potato tuber cv. Atlantic.

2) cv. Spunta: Area coverage (%) of lesion produced by the isolates on potato tuber slices cv. Spunta ranging from 5 to 81%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%. Group 1 had the smallest area and composed of 33 isolates whereas group 4 had the largest area and composed of 3 isolates (Table 20). Mean, median and mode of lesion area value were 37.00, 33.00 and 17.83, respectively. The values had positive skewed distribution.

c. Tomato leaflets

1) cv. Delta: Area coverage (%) of lesion produced by the isolates on tomato leaflets cv. Delta ranging from 3 to 84%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%. Group 1 had the smallest area and composed of 39 isolates whereas group 4 had the largest area and composed of 10 isolates (Table 20). Mean, median and mode of lesion area value were 55.86, 50.00 and 59.00, respectively. The values had negative skewed distribution.

2) cv. Seeda: Area coverage (%) of lesion produced by the isolates on tomato leaflets cv. Seeda ranging from 2 to 67%. The isolates were divided using lesion area value to 4 groups; 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4

= 76.00-100.00%. Group 1 had the smallest area and composed of 102 isolates whereas group 4 had the largest area and no isolates contained (Table 20). Mean, median and mode of lesion area value were 17.67, 17.00 and 8.13, respectively. The values had positive skewed distribution.

1.3.3 Spore capacity

a. Potato leaflets

1) cv. Atlantic: Number of sporangia produced per cm^2 of lesion by the isolates on potato leaflets cv. Atlantic ranging from 22 to 2,733 sporangia/cm^2 of lesion. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion. Group 1 produced small number of sporangia and composed of 124 isolates whereas group 3 produced numerous numbers of sporangia and composed of 2 isolates (Table 21). Mean, median and mode of spore capacity value were 355.00, 117.00 and 33.00, respectively. The values had positive skewed distribution.

2) cv. Spunta: Number of sporangia produced per cm^2 of lesion by the isolates on potato leaflets cv. Spunta ranging from 33 to 2,056 sporangia/cm^2 of lesion. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion. Group 1 produced small number of sporangia and composed of 131 isolates whereas group 3 produced numerous numbers of sporangia and composed of 1 isolate collected from Chedi Mae Krua Sub-District (Table 21). Mean, median and mode of spore capacity value were 292.00, 190.00 and 33.00, respectively. The values had positive skewed distribution.

Table 19 Number of *Phytophthora infestans* isolates collected in different regions of northern Thailand and classified into each group of infection frequency

Province[a]	District	Sub-District	Number of isolate in group based on infection frequency[b, c]																	
			Potato leaflet						Potato tuber						Tomao leaflet					
			Atlantic			Spunta			Atlantic			Spunta			Delta			Seeda		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CM	Fa	-	0	1	3	0	0	4	0	0	4	0	0	4	0	0	4	0	1	4
	SS	-	0	0	3	1	2	0	0	0	3	0	0	3	0	0	3	0	0	2
	SS	CMK	0	4	33	1	12	24	0	0	35	0	0	35	0	1	36	1	4	32
	SS	MFK	0	2	21	2	3	18	0	0	25	0	0	25	0	0	22	1	2	19
	SS	NH	1	1	45	3	10	34	0	0	46	0	0	46	0	3	43	0	6	40
	Ph	LK	0	1	4	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5
TK	PP	RTP	0	1	12	0	3	10	0	0	13	0	0	13	0	1	12	0	0	13
		Total	1	10	121	7	30	95	0	0	131	0	0	131	0	5	125	2	13	115
					132			132			131			131			130			130

[a] Isolate sources : Province; CM = Chiang Mai, T = Tak : District; F = Fang, SS = San Sai, Ph = Phrao, PP = Phop Phra
: Sub-District; NH = Nong Han, MFK = Mae Faek Mai, CMK = Chedi Mae Krua, LK = Long Khot, RTP = Ruam Thai Phatthana : - = absent

[b]Chi-square is used to analysis the relation in the aggressiveness and the source of isolate.

[c]Group base on infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

Table 20 Number of *Phytophthora infestans* isolates collected in different regions of northern Thailand and classified into each group of lesion area

Province[a]	District	Sub-District	Number of isolate in group based on lesion area[b, c]																							
			Potato leaflet								Potato tuber								Tomato leaflet							
			Atlantic				Spunta				Atlantic				Spunta				Delta				Seeda			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
CM	Fa	-	0	1	0	3	0	0	0	4	0	4	0	0	0	3	1	0	1	1	2	0	4	0	0	0
	SS	-	0	0	0	3	0	2	1	0	0	2	0	1	0	2	0	1	1	1	1	0	3	0	0	0
	SS	CMK	0	2	5	30	0	6	10	21	9	17	7	4	12	20	5	0	6	10	19	2	29	8	0	0
	SS	MFK	0	1	3	19	0	4	9	10	3	11	8	1	7	11	5	0	4	5	11	2	18	4	0	0
	SS	NH	1	0	15	31	3	2	16	26	11	22	10	3	9	28	7	2	20	8	14	4	36	5	5	0
	Ph	LK	0	0	3	2	0	0	1	4	0	1	4	0	1	3	1	0	0	1	3	1	3	1	1	0
TK	PP	RTP	0	0	4	9	1	3	2	7	4	8	0	1	4	8	1	0	7	1	4	1	9	4	0	0
		Total	1	4	30	97	4	17	39	72	27	65	29	10	33	75	20	3	39	27	54	10	102	22	6	0
						132				132				131				131				130				130

[a] Isolate sources : Province; CM = Chiang Mai, T = Tak : District; F = Fang, SS = San Sai, Ph = Phrao, PP = Phop Phra

: Sub-District; NH = Nong Han, MFK = Mae Faek Mai, CMK = Chedi Mae Krua, LK = Long Khot, RTP = Ruam Thai Phatthana : - = absent

[b]Chi-square is used to analysis the relation in the pathogenicity and the source of isolate.

[c]Group based on lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

Table 21 Number of *Phytophthora infestans* isolates collected in different regions of northern Thailand and classified into each group of spore capacity

Province[a]	District	Sub-District	Number of isolate in group based on spore capacity[b, c]																	
			Potato leaflet						Potato tuber						Tomato leaflet					
			Atlantic			Spunta			Atlantic			Spunta			Delta			Seeda		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
CM	Fa	-	4	0	0	4	0	0	4	0	0	4	0	0	4	0	0	4	0	0
	SS	-	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0	3	0	0
	SS	CMK	36	1	0	36	0	1	32	4	1	29	6	2	37	0	0	37	1	1
	SS	MFK	21	2	0	23	0	0	21	2	0	19	4	0	22	0	0	22	0	0
	SS	NH	46	0	1	47	0	0	42	4	0	39	7	0	43	1	2	44	0	0
	Ph	LK	2	2	1	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0
TK	PP	RTP	12	1	0	13	0	0	11	2	0	9	4	0	12	1	0	13	0	0
		Total	124	6	2	131	0	1	118	12	1	108	21	2	126	2	2	128	1	1
					132			132			131			131			130			130

[a] Isolate sources : Province; CM = Chiang Mai, T = Tak : District; F = Fang, SS = San Sai, Ph = Phrao, PP = Phop Phra

: Sub-District; NH = Nong Han, MFK = Mae Faek Mai, CMK = Chedi Mae Krua, LK = Long Khot, RTP = Ruam Thai Phatthana : - = absent

Table 21 (continued)

[b]Chi-square is used to analysis the relation in the pathogenicity and the source of isolate.

[c]Group based on spore capacity (SC) on potato and tomato leaflets; 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion, on potato tuber cv. Atlantic; 1 = 0.00-4000.00, 2 = 4001.00-8000.00, and 3 = 8001.00-12000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick), and on potato tuber cv. Spunta; 1 = 0.00-3000.00, 2 = 3001.00-6000.00, and 3 = 6001.00-9000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick).

b. Potato discs

1) cv. Atlantic: Number of sporangia produced per tuber disc (0.7 cm diameter, 0.5 cm thick) by the isolates on potato discs cv. Atlantic ranging from 222 to 11111 sporangia/ tuber disc. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-4000.00, 2 = 4001.00-8000.00, and 3 = 8001.00-12000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick). Group 1 produced small number of sporangia and composed of 118 isolates whereas group 3 produced numerous numbers of sporangia and composed of 1 isolate collected from Chedi Mae Krua Sub-District (Table 21). Mean, median and mode of spore capacity value were 2162.00, 1797.00 and 2889.00, respectively. The values had positive skewed distribution.

2) cv. Spunta: Number of sporangia produced per tuber disc (0.7 cm diameter, 0.5 cm thick) by the isolates on potato discs cv. Spunta ranging from 111 to 6815 sporangia/ tuber disc. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-3000.00, 2 = 3001.00-6000.00, and 3 = 6001.00-9000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick). Group 1 produced small number of sporangia and composed of 108 isolates whereas group 3 produced numerous numbers of sporangia and composed of 2 isolates collected from Chedi Mae Krua Sub-District (Table 21). Mean, median and mode of spore capacity value were 1,816.00, 1,333.00 and 370.00, respectively. The values had positive skewed distribution.

c. Tomato leaflets

1) cv. Delta: Number of sporangia produced per cm^2 of lesion by the isolates on tomato leaflets cv. Delta ranging from 180 to 2178 sporangia/cm^2 of lesion. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion. Group 1 produced small number of sporangia and composed of 126 isolates whereas group 3 produced numerous numbers of sporangia and composed of 2 isolates

collected from Nong Han Sub-District (Table 21). Mean, median and mode of spore capacity value were 635.00, 581.00 and 489.00, respectively. The values had positive skewed distribution.

2) cv. Seeda: Number of sporangia produced per cm^2 of lesion by the isolates on tomato leaflets cv. Seeda ranging from 148 to 2,100 sporangia/cm^2 of lesion. The isolates were divided using spore capacity value to 3 groups; 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion. Group 1 produced small number of sporangia and composed of 128 isolates whereas group 3 produced numerous numbers of sporangia and composed of 1 isolate collected from Chedi Mae Krua Sub-District (Table 21). Mean, median and mode of spore capacity value were 544.00, 476.00 and 148.00, respectively. The values had positive skewed distribution.

1.4 Analysis of relation between the aggressiveness of *Phytophthora infestans* Thai isolates and another factors

1.4.1 Aggressiveness of Thai isolates collected in different regions of northern Thailand

The relationship between groups of aggressiveness parameters and sources of the isolates was analyzed by Chi-square test at P=0.01. The aggressiveness parameters had no relation with the sources of isolates except spore capacity on both potato leaflet cultivars; Asymp. Sig. were 0.001 and 0.000 for Atlantic and Spunta, respectively (Table 22 and Appendix Table E5-21). Capability on infection of isolates on potato discs cv. Atlantic and Spunta could not be analyzed because it was only 1 group of infection frequency.

1.4.2 Aggressiveness of Thai isolates Thai isolates obtained from different sizes of micro-morphology

The relationship between groups of the aggressiveness parameter (lesion area) on potato leaflets, cv. Atlantic and Spunta, and the sizes of micro-morphology were analyzed by Chi-square test at *P*=0.01. The relation was found between the lesion area coverage on potato leaflet cv. Spunta and the size of micro-morphology showing Asymp. Sig. was 0.001 (Table 22 and Appendix Table E22-23). No relation was found on potato leaflet cv. Atlantic.

1.5 Clusters of *Phytophthora infestans* Thai isolates classified by multi-characteristic of aggressiveness parameters

P. infestans Thai isolates were classified by using all 3 parameters of the aggressiveness (Table 23-28). Diversities of the isolates using aggressiveness parameters were analyzed by Shannon and Evenness indices.

1.5.1 Potato leaflets cv. Atlantic

Eight clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on potato leaflets cv. Atlantic (Table 23). The cluster 6 contained the most number of isolates (68.9% of Thai isolates) while the cluster 1 contained the least number of isolates (0.8% of Thai isolates). The isolates contained in cluster 6 produced the highest infections and the largest lesion area, but low sporulation. Shannon and Evenness indices of the clusters of aggressiveness parameters on potato leaflets cv. Atlantic were 1.079 and 0.591, respectively.

Table 22 Chi-square value for the relationship analysis between groups of aggressiveness and sources or micro-morphological size of the isolates at *P*=0.01

Analysis	Plant	Cultivar	Chi-square[a]	Asymp. Sig (2-tailed)
Infection frequency	Potato leaflet	Atlantic	7.378	0. 832
x Source		Spunta	17.379	0. 136
	Potato tuber	Atlantic	-	-
		Spunta	-	-
	Tomato leaflet	Delta	2.901	0. 821
		Seeda	6.060	0. 913
Lesion area	Potato leaflet	Atlantic	22.151	0.225
x Source		Spunta	24.854	0.129
	Potato tuber	Atlantic	25.877	0. 103
		Spunta	21.746	0. 243
	Tomato leaflet	Delta	17.871	0. 464
		Seeda	15.097	0. 236
Spore capacity	Potato leaflet	Atlantic	32.036	0. 001
x Source		Spunta	98.889	0. 000
	Potato tuber	Atlantic	4.555	0. 971
		Spunta	9.641	0. 647
	Tomato leaflet	Delta	8.219	0. 768
		Seeda	4.740	0. 966
Lesion area	Potato leaflet	Atlantic	73.370	0. 596
x Micro-morphological size		Spunta	146.260	0. 001

[a] "-" = No statistics are computed because infection frequency groups of potato tuber cv. Atlantic and Spunta are constant.

1.5.2 Potato leaflets cv. Spunta

Ten clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on potato leaflets cv. Spunta (Table 24). The cluster 9 contained the most number of isolates (53.8% of Thai isolates) while the cluster 3, 6, 7, and 10 contained the least number of isolates (0.8% of Thai isolates for each cluster). The isolates contained in cluster 9 produced the highest infections and the largest lesion area, but low sporulation. Shannon and Evenness indices of the clusters of aggressiveness parameters on potato leaflets cv. Spunta were 1.443 and 0.627, respectively.

1.5.3 Potato tuber cv. Atlantic

Nine clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on potato tuber cv. Atlantic (Table 25). The cluster 2 contained the most number of isolates (44.3% of Thai isolates) while the cluster 8 and 9 contained the least number of isolates (0.8% of Thai isolates for each cluster). Isolate 52 in cluster 9 produced high infections and sporulation, and large lesion area. Shannon and Evenness indices of the clusters of aggressiveness parameters on potato tuber cv. Atlantic were 1.540 and 0.701, respectively.

1.5.4 Potato tuber cv. Spunta

Ten clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on potato tuber cv. Spunta (Table 26). The cluster 2 contained the most number of isolates (46.6% of Thai isolates) while the cluster 8, 9, and 10 contained the least number of isolates (0.8% of Thai isolates for each cluster). Isolate 52 in cluster 10 produced high infections and sporulation, and large lesion area. Shannon and Evenness indices of the clusters of aggressiveness parameters on potato tuber cv. Spunta were 1.544 and 0.671, respectively.

1.5.5 Tomato leaflets cv. Delta

Nine clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on tomato leaflets cv. Delta (Table 27). The cluster 4 contained the most number of isolates (40.8% of Thai isolates) while the cluster 6, 7, 8, and 9 contained the least number of isolates (0.8% of Thai isolates for each cluster). The isolates contained in cluster 5 produced the highest infections and the largest lesion area, but low sporulation. Shannon and Evenness indices of the clusters of aggressiveness parameters on tomato leaflets cv. Delta were 1.491 and 0.679, respectively.

1.5.6 Tomato leaflets cv. Seeda

Seven clusters of *P. infestans* Thai isolates were classified by aggressiveness parameters on tomato leaflets cv. Seeda (Table 28). The cluster 3 contained the number of isolates (66.2% of Thai isolates) while the cluster 1, 6 and 7 contained the least number of isolates (0.8% of Thai isolates for each cluster). The isolates contained in cluster 5 produced the highest infections and the large lesion area, but low sporulation. Shannon and Evenness indices of the clusters of aggressiveness parameters on tomato leaflets cv. Seeda were 1.075 and 0.552, respectively.

1.6 Diversity on the pathogenicity of *Phytophthora infestans* population

The pathogenicity diversity of *P. infestans* population was revealed by Shannon and Evenness values from aggressiveness parameters on each host. The population showed the higher diversity after infection on potato tuber cv. Atlantic and Spunta than another hosts. For contrast, diversity on the pathogeneicity of the population was low after infection on potato leaflet cv. Atlantic and tomato leaflet cv. Seeda.

Table 23 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on potato leaflets cv. Atlantic

	Group			Isolate		
Cluster	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	1	1	1	1	0.8	110
2	2	2	1	4	3.0	89, 104, 107, 135
3	2	3	1	4	3.0	23, 48, 94, 139
4	2	4	1	2	1.5	14, 83
5	3	3	1	22	16.7	27, 35, 91, 96, 98, 99, 100, 101, 102,103, 105, 106, 111, 112, 113, 115,118, 119, 121, 128, 129, 131
6	3	4	1	91	68.9	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16, 17, 18, 19, 20, 22, 24, 25, 26, 28, 29, 30, 31, 32, 33, 34, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 49, 50, 51, 52, 54, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 88, 92, 93, 95, 108, 109, 114, 116, 117, 120, 126, 127, 132, 133, 134, 136
7	3	3	2	4	3.0	97, 123, 138, 140
8	3	4	2	4	3.0	21, 125, 122, 137
			Total	132	100	
			Shannon index	1.079		
			Evenness index	0.519		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion.

Table 24 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on potato leaflets cv. Spunta

Cluster	Group			Isolate		
	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	1	1	1	3	2.3	58, 65, 134
2	1	2	1	4	3.0	2, 28, 31, 98
3	2	1	1	1	0.8	126
4	2	2	1	12	9.1	3, 4, 29, 33, 45, 60, 69, 87, 97, 115, 116, 118
5	2	3	1	15	11.4	7, 9, 20, 26, 27, 50, 57, 67, 68, 72, 102,106, 109, 110, 127
6	2	4	1	1	0.8	89
7	3	2	1	1	0.8	101
8	3	3	1	23	17.4	8, 11, 15, 17, 21, 34, 41, 42, 63, 66, 70, 75, 81, 82, 85, 91, 93, 103, 107, 108,123, 132, 136
9	3	4	1	71	53.8	1, 5, 6, 10, 13, 14, 16, 18, 19, 22, 23, 24, 25, 30, 32, 35, 36, 37, 38, 39, 43, 44, 46, 47, 48, 49, 51, 52, 54, 56, 59, 62, 64, 73, 74, 76, 77, 78, 79, 80, 83, 84, 86, 88, 92, 94, 95, 96, 99, 100, 104, 105, 111, 112, 113, 114, 117, 119, 120, 121, 122, 125, 128, 129, 131, 133, 135, 137, 138, 139, 140
10	2	3	3	1	0.8	40
			Total	132	100	
			Shannon index	1.443		
			Evenness index	0.627		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion.

Table 25 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on potato tubers cv. Atlantic

Cluster	Group			Isolate		
	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	3	1	1	25	19.1	3, 4, 5, 15, 16, 18, 20, 23, 26, 32, 40, 43, 46, 48, 65, 70, 72, 81, 88, 91, 92, 95, 110, 126, 133
2	3	2	1	58	44.3	1, 2, 6, 7, 8, 9, 10, 11, 13, 14, 17, 19, 21, 22, 28, 30, 36, 37, 38, 39, 42, 47, 51, 57, 59, 66, 69, 73, 74, 75, 76, 77, 79, 86, 89, 93, 94, 96, 97, 99, 100, 101, 102, 104, 107, 108, 111, 113, 116, 118, 119, 121, 123, 128, 129, 131, 132, 140
3	3	3	1	26	19.8	25, 27, 31, 35, 41, 49, 50, 56, 60, 63, 64, 67, 82, 83, 85, 98, 103, 112, 114, 117, 122, 125, 136, 137, 138, 139
4	3	4	1	9	6.9	34, 80, 84, 87, 105, 109, 115, 120, 135
5	3	1	2	2	1.5	58, 62
6	3	2	2	7	5.3	24, 29, 45, 54, 68, 78, 106
7	3	3	2	2	1.5	33, 134
8	3	4	2	1	0.8	127
9	3	3	3	1	0.8	52
			Total	131	100	
			Shannon index	1.540		
			Evenness index	0.701		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-4,000.00, 2 = 4,001.00-8,000.00, and 3 = 8,001.00-12,000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick).

Table 26 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on potato tubers cv. Spunta

Cluster	Group			Isolate		
	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	3	1	1	28	21.4	1, 2, 3, 4, 8, 15, 20, 23, 24, 38, 48, 59, 72, 74, 91, 94, 105, 108, 110, 116, 119, 121, 123, 128, 129, 131, 133, 137
2	3	2	1	61	46.6	5, 6, 7, 9, 11, 13, 16, 17, 18, 19, 21, 22, 26, 28, 31, 32, 35, 36, 37, 39, 40, 41, 47, 50, 56, 57, 60, 67, 73, 75, 77, 79, 80, 81, 82, 83, 85, 86, 88, 89, 92, 95, 100, 101, 102, 103, 104, 107, 111, 112, 115, 117, 118, 120, 125, 126, 132, 134, 136, 138, 139
3	3	3	1	17	13.0	10, 27, 30, 34, 63, 64, 69, 70, 97, 98, 99, 106, 109, 113, 122, 135, 140
4	3	4	1	2	1.5	42, 87
5	3	1	2	5	3.8	14, 43, 45, 84, 114
6	3	2	2	13	9.9	25, 29, 46, 49, 54, 58, 62, 65, 68, 76, 78, 93, 96
7	3	3	2	2	1.5	33, 66
8	3	4	2	1	0.8	51
9	3	2	3	1	0.8	127
10	3	3	3	1	0.8	52
			Total	131	100	
			Shannon index	1.544		
			Evenness index	0.671		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-3,000.00, 2 = 3,001.00-6,000.00, and 3 = 6,001.00-9,000.00 sporangia/ tuber discs (0.7 cm diameter x 0.5 cm thick).

Table 27 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on tomato leaflets cv. Delta

Cluster	Group			Isolate		
	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	2	1	1	4	3.1	81, 88, 92, 108
2	3	1	1	32	24.6	7, 9, 10, 14, 30, 59, 60, 67, 69, 72, 74, 75, 79, 82, 83, 86, 93, 94, 96, 97, 99, 101, 105, 110, 111, 113, 116, 117, 118, 119, 121, 126
3	3	2	1	27	20.8	8, 16, 17, 22, 28, 36, 39, 56, 57, 63, 80, 84, 85, 89, 95, 98, 100, 103, 104, 107, 109, 112, 114, 115, 120, 122, 139
4	3	3	1	53	40.8	1, 2, 3, 4, 5, 6, 11, 13, 15, 18, 19, 23, 24, 25, 26, 27, 29, 31, 32, 33, 34, 35, 38, 40, 41, 42, 43, 45, 46, 47, 48, 52, 54, 58, 64, 65, 66, 68, 70, 73, 76, 77, 78, 87, 125, 127, 131, 132, 133, 134, 137, 138, 140
5	3	4	1	10	7.7	20, 37, 49, 50, 51, 123, 128, 129, 135, 136
6	3	1	2	1	0.8	102
7	3	3	2	1	0.8	62
8	2	1	3	1	0.8	91
9	3	1	3	1	0.8	106
			Total	130	100	
			Shannon index	1.491		
			Evenness index	0.679		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion.

Table 28 Clusters of *Phytophthora infestans* Thai isolates classified by aggressiveness parameters on tomato leaflets cv. Seeda

Cluster	Group			Isolate		
	IF[a]	LA[b]	SC[c]	Number	Percentage	Name
1	1	1	1	2	1.5	93, 107
2	2	1	1	12	9.2	72, 82, 83, 84, 87, 92, 98, 100, 103, 109, 111, 112
3	3	1	1	86	66.2	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 37, 39, 40, 42, 45, 47, 48, 49, 50, 51, 52, 54, 63, 64, 68, 70, 73, 74, 75, 76, 77, 78, 79, 80, 81, 85, 86, 89, 91, 94, 95, 96, 97, 99, 101, 102, 104, 105, 108, 110, 114, 115, 116, 117, 118, 119, 120, 121, 122, 131, 132, 133, 134, 135, 136, 137, 138
4	3	2	1	22	16.9	15, 18, 34, 38, 41, 43, 46, 56, 58, 62, 65, 66, 67, 69, 88, 123, 125, 126, 127, 128, 129, 139
5	3	3	1	6	4.6	35, 36, 57, 59, 60, 140
6	3	1	2	1	0.8	113
7	2	1	3	1	0.8	106
			Total	130	100	
			Shannon index	1.075		
			Evenness index	0.552		

[a]Group of infection frequency (IF); 1 = 0.10-0.40, 2 = 0.41-0.70, and 3 = 0.71-1.00.

[b]Group of lesion area coverage (LA); 1 = 0.00-25.00, 2 = 26.00-50.00, 3 = 51.00-75.00, and 4 = 76.00-100.00%.

[c]Group of spore capacity (SC); 1 = 0.00-1000.00, 2 = 1001.00-2000.00, and 3 = 2001.00-3000.00 sporangia/cm^2 of lesion.

2. Host Range Test of *Phytophthora infestans* Thai-isolates

P. infestans certainly caused disease on potato leaflets/tubers and tomato leaflets/fruits and produced lesion and sporangia on both infected plants. As for eggplant, chilli pepper and petunia leaves were expressed the lesion at 9.67, 3.50 and 2.67 days after inoculation, respectively. Most of lesion on these plants showed dark-brown water soaked lesion at the inoculation point. Pea eggplant leaves, eggplant fruits, Thai green eggplant leaves, tobacco leaves, night-blooming Jessamine leaves, yesterday-today-and-tomorrow leaves, ground cherry, Thorn apple and water morning glory had no lesions after inoculation (Table 29 and Fig. 11).

Table 29 The incubation period and number of leaves or leaflets or fruits showing lesion after inoculation of *Phytophthora infestans*

Plant species	Incubation period (days)[a]	Number of symptomatic leaves or leaflets or fruits[b]
Pea eggplant leaves	-	-
Eggplant leaves	9.67	6
Eggplant fruits	-	-
Thai green eggplant leaves	-	-
Tobacco leaves	-	-
Chilli pepper leaves	3.50	5
Chilli pepper fruits	-	-
Thorn apple	-	-
Tomato leaflets	6.83	6
Tomato fruits	3.67	6
Petunia leaves	2.67	6
Potato leaflets	2.80	6
Night-blooming Jessamine leaves	-	-
Yesterday-today-and-tomorrow leaves	-	-
Ground cherry	-	-
Water morning glory	-	-

[a]Average of incubation period obtained from 6 leaves or leaflets or fruits which used for individual plant species. ‘-’ = no symptom was expressed.

[b]Number of leaves or leaflets or fruits that showed lesion when inoculated with *P. infestans* isolate 39.

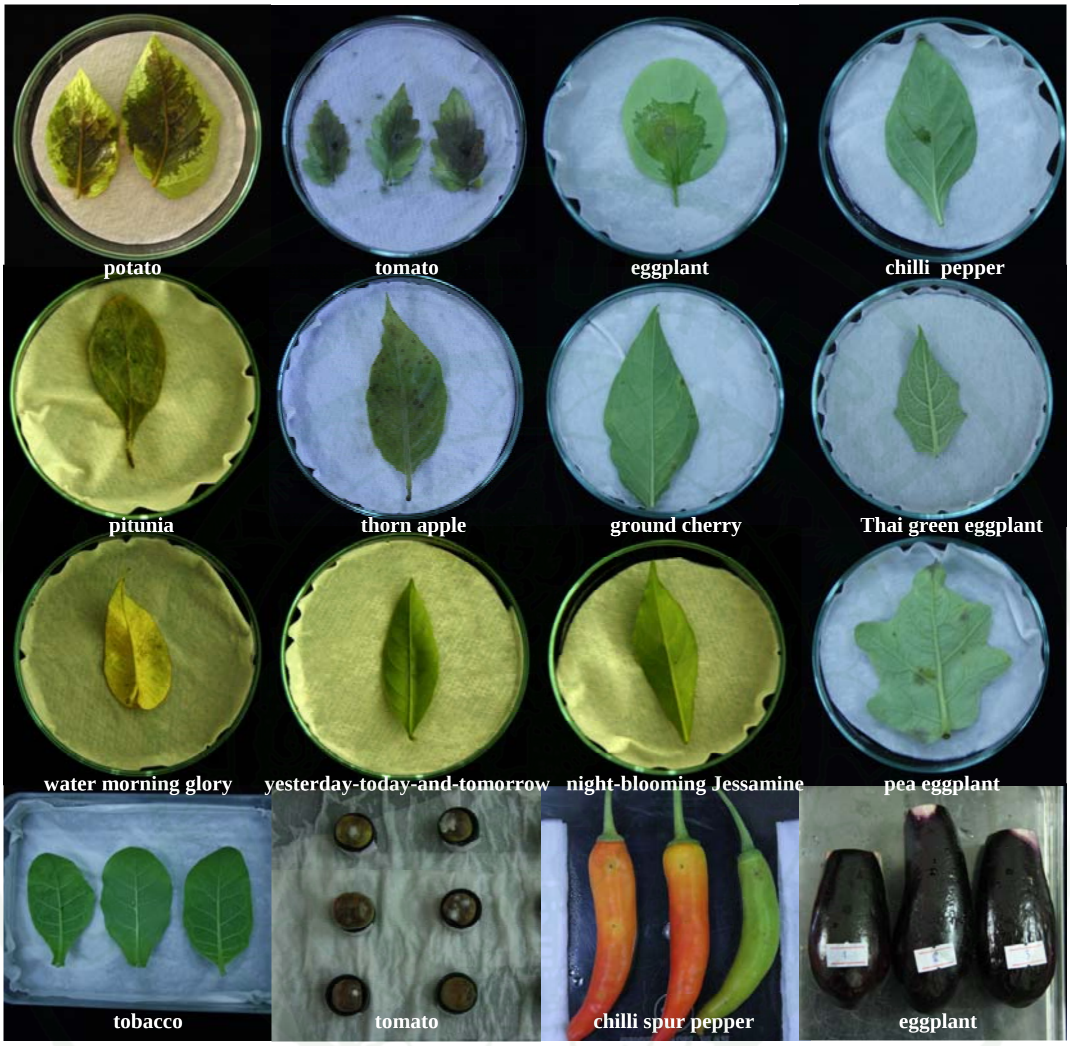

Figure 11 Host range test of *Phytophthora infestans*-Thai isolate 39. Symptom on potato leaflets at 5 days after incubation (dai); tomato leaflets at 7 dai; leaves of eggplant, chilli pepper, petunia, thorn apple ground cherry, Thai green eggplant, water morning glory, yesterday-today-and-tomorrow, night-blooming Jessamine, pea eggplant, and tobacco and fruits of tomato, chilli pepper and eggplant at 15 dai.

Chapter 4
Genetic distribution and characteristics of *Phytophthora infestans*-Thai isolates

1. Mating Type of *Phytophthora infestans*-Thai isolates

One hundred thirty two Thai isolates were paired with the known mating type testers, A1 and A2, and itself on rye B agar for 2 weeks and revealed that all isolates formed oospore with A2 tester but not A1 tester. None of Thai isolate was self fertile. All Thai isolates were mating type A1 (Table 30).

2. Metalaxyl sensitivity of *Phytophthora infestans*-Thai isolates

2.1 Metalaxyl sensitivity test

Of 132 isolates tested, 120 isolates (90.9%) were intermediately resistant. Only 12 isolates (9.1%) were sensitive to metalaxyl (Table 30 and 31). The isolates collected from Chedi Mae Krua and Nong Han Sub-Districts showed 100% intermediate to metalaxyl while isolates from Long Khot showed 100% sensitive to metalaxyl. The isolates collected from Mae Faek Mai and Ruam Thai Phatthana Sub-Districts showed that intermediate isolates higher 10.5 and 1.6 folds than sensitive isolates, respectively.

2.2 The relations between the metalaxyl sensitivity of the isolates and source of isolates or size of reproductive propagules or aggressiveness (lesion area)

2.2.1 The relation between the metalaxyl sensitivity of the isolates and source of isolates

Chi-squared analysis indicated a highly significant (P = 0.01) relation of metalaxyl resistance with sources of isolates (Table 32 and Appendix Table E24). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 72.674 and 0.000.

Table 30 Metalaxyl sensitivity, mating type and mtDNA haplotype of *Phytophthora infestans* Thai isolates

Hyphal tip culture code	Accession number	Metalaxyl sensitivity[a]	Mating type	mtDNA haplotype
1	CMSS1-05	I	A1	IIa
2	CMSS2-10	S	A1	IIa
3	CMSS2-01	I	A1	IIa
4	CMSS1-06	I	A1	IIa
5	TKPP1-06	S	A1	IIa
6	CMFa0-05	I	A1	IIa
7	CMSS2-04	I	A1	IIa
8	CMSS2-05	I	A1	IIa
9	CMSS0-03	I	A1	IIa
10	CMFa0-03	I	A1	IIa
11	CMSS1-07	I	A1	IIa
12	CMSS2-16	nd	nd	nd
13	CMSS3-16	I	A1	IIa
14	TKPP1-07	S	A1	IIa
15	CMSS2-07	I	A1	IIa
16	CMSS1-01	I	A1	IIa
17	TKPP1-04	S	A1	IIa
18	CMSS3-08	I	A1	IIa
19	CMSS1-04	I	A1	IIa
20	CMSS3-13	I	A1	IIa
21	CMSS2-03	S	A1	IIa
22	CMSS2-15	I	A1	IIa
23	CMSS2-08	I	A1	IIa
24	CMSS1-08	I	A1	IIa
25	CMSS3-15	I	A1	IIa
26	CMSS1-02	I	A1	IIa
27	CMSS3-10	I	A1	IIa
28	CMSS0-06	I	A1	IIa
29	TKPP1-03	S	A1	IIa
30	CMSS3-05	I	A1	IIa
31	CMSS2-02	I	A1	IIa
32	CMSS3-09	I	A1	IIa
33	CMSS2-23	I	A1	IIa
34	CMSS3-24	I	A1	IIa
35	CMSS3-26	I	A1	IIa
36	CMSS3-42	I	A1	IIa
37	CMSS2-24	I	A1	IIa
38	CMSS1-31	I	A1	IIa
39	CMSS1-21	I	A1	IIa

Table 30 (Continued)

Hyphal tip culture code	Accession number	Metalaxyl sensitivity[a]	Mating type	mtDNA haplotype
40	CMSS1-32	I	A1	IIa
41	CMSS1-35	I	A1	IIa
42	CMSS3-37	I	A1	IIa
43	CMSS1-19	I	A1	IIa
44	CMSS3-27	I	A1	IIa
45	CMSS1-30	I	A1	IIa
46	CMSS1-37	I	A1	IIa
47	CMSS1-03	I	A1	IIa
48	CMSS1-12	I	A1	IIa
49	CMSS3-34	I	A1	IIa
50	CMSS1-16	I	A1	IIa
51	CMSS3-36	I	A1	IIa
52	CMSS1-18	I	A1	IIa
54	CMSS1-40	I	A1	IIa
56	CMSS2-13	I	A1	IIa
57	CMSS3-51	I	A1	IIa
58	CMSS3-54	I	A1	IIa
59	CMSS3-45	I	A1	IIa
60	CMSS3-44	I	A1	IIa
61	CMSS1-33	nd	nd	nd
62	TKPP1-12	I	A1	IIa
63	CMSS2-19	I	A1	IIa
64	CMSS2-25	I	A1	IIa
65	CMSS3-50	I	A1	IIa
66	CMSS2-20	I	A1	IIa
67	CMSS1-33	I	A1	IIa
68	CMSS3-03	I	A1	IIa
69	CMSS1-24	I	A1	IIa
70	CMSS3-48	I	A1	IIa
72	CMSS3-06	I	A1	IIa
73	CMFa0-09	I	A1	IIa
74	CMSS2-09	I	A1	IIa
75	CMSS3-40	I	A1	IIa
76	TKPP1-02	S	A1	IIa
77	CMSS1-15	I	A1	IIa
78	CMSS2-22	I	A1	IIa
79	CMSS3-28	I	A1	IIa
80	CMSS3-29	I	A1	IIa
81	CMSS3-31	I	A1	IIa
82	CMSS2-21	I	A1	IIa

Table 30 (Continued)

Hyphal tip culture code	Accession number	Metalaxyl sensitivity[a]	Mating type	mtDNA haplotype
83	CMSS1-20	I	A1	IIa
84	CMSS1-26	I	A1	IIa
85	CMSS3-32	I	A1	IIa
86	CMSS1-29	I	A1	IIa
87	CMSS0-05	I	A1	IIa
88	TKPP1-10	I	A1	IIa
89	CMSS1-14	I	A1	IIa
91	CMSS3-46	I	A1	IIa
92	CMSS3-52	I	A1	IIa
93	CMSS2-14	I	A1	IIa
94	CMSS3-35	I	A1	IIa
95	CMSS1-36	I	A1	IIa
96	CMSS3-53	I	A1	IIa
97	CMSS1-09	I	A1	IIa
98	CMSS1-13	I	A1	IIa
99	TKPP1-05	I	A1	IIa
100	CMSS3-47	I	A1	IIa
101	TKPP1-08	I	A1	IIa
102	CMSS3-11	I	A1	IIa
103	CMSS2-18	I	A1	IIa
104	CMFa0-07	I	A1	IIa
105	TKPP1-09	I	A1	IIa
106	CMSS3-01	I	A1	IIa
107	CMSS1-22	I	A1	IIa
108	CMSS1-25	I	A1	IIa
109	CMSS1-41	I	A1	IIa
110	CMSS3-39	I	A1	IIa
111	CMSS3-38	I	A1	IIa
112	CMSS3-33	I	A1	IIa
113	CMSS3-22	I	A1	IIa
114	CMSS1-27	I	A1	IIa
115	CMSS1-17	I	A1	IIa
116	TKPP1-11	I	A1	IIa
117	CMSS3-41	I	A1	IIa
118	CMSS3-23	I	A1	IIa
119	CMSS3-21	I	A1	IIa
120	CMSS3-14	I	A1	IIa
121	CMSS3-25	I	A1	IIa
122	CMSS3-49	I	A1	IIa
123	TKPP1-01	I	A1	IIa

Table 30 (Continued)

Hyphal tip culture code	Accession number	Metalaxyl sensitivity[a]	Mating type	mtDNA haplotype
125	CMSS2-11	I	A1	IIa
126	TKPP1-13	I	A1	IIa
127	CMSS1-39	I	A1	IIa
128	CMSS1-42	I	A1	IIa
129	CMSS3-20	I	A1	IIa
131	CMSS2-06	I	A1	IIa
132	CMSS3-07	I	A1	IIa
133	CMSS1-28	I	A1	IIa
134	CMSS3-04	I	A1	IIa
135	CMSS2-17	I	A1	IIa
136	CMPh4-01	S	A1	IIa
137	CMPh4-02	S	A1	IIa
138	CMPh4-03	S	A1	IIa
139	CMPh4-04	S	A1	IIa
140	CMPh4-05	S	A1	IIa
US-1	WI 94-1	S	A1	Ib
US-7	BG-8	R	A2	Ia
US-11	US 980008	R	A1	IIb

[a]Resistant (R) = both 5 and 100 μg/ml ≥ 40% growth at 0 μg/ml. Intermediate (I) = 5 μg/ml ≥ 40% growth of 0 μg/ml. Sensitive (S) = both 5 and 100 μg/ml < 40% growth at 0 μg/ml. nd = not determined.

2.2.2 The relations between the metalaxyl sensitivity of the isolates and size of reproductive propagules

a. Size of L:B ratio: Chi-squared analysis (P = 0.01) indicated that the levels of metalaxyl sensitivities were not related to size of sporangia (L:B ratio) (Table 32 and Appendix Table E25). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 8.701 and 0.013.

b. Size of pedicel: Chi-squared analysis (P = 0.01) indicated that the levels of metalaxyl sensitivities were not related to size of pedicel (Table 32 and

Appendix Table E26). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 1.640 and 0.440.

c. Size of oospore: Chi-squared analysis (P = 0.01) indicated that the levels of metalaxyl sensitivities were not related to size of oospore (Table 32 and Appendix Table E27). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 3.538and 0.170.

2.3.3 The relations between the metalaxyl sensitivity of the isolates and aggressiveness (lesion area)

The relations between metalaxyl sensitivity of among population and the ability to produce lesion area (LA) on both the potato leaflets cv. Atlantic and Spunta were analyzed by Chi Square at P=0.01.

a. Potato leaflets cv. Atlantic: The ability to produce lesion area of isolates on potato leaflets cv. Atlantic had no relationship with metalaxyl sensitivity of among population (Appendix Table E28). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 0.534 and 0.534.

b. Potato leaflets cv. Spunta: The ability to produce lesion area of isolates on potato leaflets cv. Spunta had no relationship with metalaxyl sensitivity of among population (Appendix Table E29). Pearson Chi-square and Asymp. Sig. (2-tailed) values were 0.674 and 0.879.

2.3 The purity of metalaxyl used in metalaxyl sensitivity test

The comparative test on sensitivities of *P. infestans* to metalaxyl technical (98.1%) and commercial (25%) grade amended in rye A agar showed that the 7 representative Thai- and 3 US-isolates had similar levels of sensitivities to metalaxyl on both rye A agar supplemented with technical or commercial grade (Table 33). The growths (%) between media supplemented with technical or commercial grade at 5 and 100 ppm were compared using Z-test revealed that the growth of *P. infestans* had no difference in both amended media (Table 33 and Appendix Table E30-31).

3. Mitochondrial DNA haplotypes of *Phytophthora infestans*-Thai isolates mtDNA haplotype

The 132- PCR products from Thai isolates were used in this study. The 890 bp-PCR products amplified from the P2 primer were cut with *Msp*I providing the pattern of DNA bands at the size of 147, 203 and 890 bp (Fig. 12). The 964 bp-PCR products amplified from the P4 primer were cut with *Eco*RI providing the pattern of DNA bands at the size of 361 and 603 bp (Fig. 13).

One mitochondrial haplotype was detected among the 132 Thai-isolates tested (Table 30). Haplotype IIa predominated in the all Thai isolates (Fig. 14). US-1, US-7 and US-11 were haplotype Ib, Ia and IIb, respectively.

Table 31 Number of northern Thai isolates of *Phytophthora infestans* sensitive (S) and intermediate (I) to metalaxyl derived from various source

Province	District	Sub-District[a]	Number of isolates[b]	
			Sensitive	Intermidiate
Chiang Mai	Fang	-	0	4
	San Sai	-	0	3
	San Sai	Chedi Mae Krua	0	37
	San Sai	Mae Faek Mai	2	21
	San Sai	Nong Han	0	47
	Phrao	Long Khot	5	0
Tak	Phob Phra	Ruam Thai Phatthana	5	8
		Total	12	120

[a]"-" = absent data.

[b]Chi-square was used to analysis the relation between the sources of isolates and the level of metalaxyl sensitivity. Resistant (R) = both 5 and 100 μg/ml $\geq$ 40% growth at 0 μg/ml. Intermediate (I) = 5 μg/ml $\geq$ 40% growth of 0 μg/ml. Sensitive (S) = both 5 and 100 μg/ml < 40% growth at 0 μg/ml.

Table 32 Number of northern Thai isolates of *Phytophthora infestans* in each group of reproductive propagules divided by size derived from sensitive and intermediate isolate to metalaxyl

Metalaxyl sensitivity[a]	Number of isolate[b]								
	Size based on L:B ratio[c]			Size based on pedicel[d]			Size based on oospore[e]		
	1	2	3	1	2	3	1	2	3
Intermidiate	6	91	23	111	8	1	3	115	2
Sensitive	2	9	1	10	2	0	1	10	1

[a]Resistant (R) = both 5 and 100 μg/ml ≥ 40% growth at 0 μg/ml. Intermediate (I) = 5 μg/ml ≥ 40% growth of 0 μg/ml. Sensitive (S) = both 5 and 100 μg/ml < 40% growth at 0 μg/ml.

[b]Chi-square was used to analysis the association in size of reproductive propagules and level of metalaxyl sensitivity at P = 0.01.

[c]Each group of sporangial (L:B ratio) represents 1 = 1.00-1.50, 2 = 1.60-2.00, and 3 = 2.10-2.50.

[d]Each group of pedicel represents 1 = 0.50-2.00, 2 = 2.10-3.50, and 3 = 3.60-5.00 μm.

[e]Each group of each oospore represents 1 = 25.00-29.00, 2 = 30.00-35.00, and 3 = 36.00-40.00 μm.

Table 33 The sensitivities of the 7 representative *Phytophthora infestans*-Thai isolates on technical grade and commercial grade of metalaxyl amended in rye A agar at 18^{o}C of incubation

Isolate	Metalaxyl (ppm)												Metalaxyl sensitivity[c]
	Technical grade[a]						Commercial grade[a]						
	Colony diameter (mm)			Growth as percent of control[b]			Colony diameter (mm)			Growth as percent of control[b]			
	0	5	100	0	5	100	0	5	100	0	5	100	
CMSS1-39	65.70	38.00	12.00	100.00	57.84	18.26	60.00	34.00	10.70	100.00	56.67	17.83	I
CMSS2-17	64.30	32.70	10.00	100.00	50.86	15.55	53.70	29.30	5.00	100.00	54.56	9.31	I
CMSS3-04	59.00	46.30	10.00	100.00	78.47	16.95	86.70	64.30	19.00	100.00	74.16	21.91	I
CMPr4-05	70.00	13.00	10.00	100.00	18.57	14.29	53.30	5.00	5.00	100.00	9.38	9.38	S
TKPP1-01	73.80	55.70	15.30	100.00	75.47	20.73	74.30	57.30	16.70	100.00	77.12	22.48	I
TKPP1-02	64.70	18.00	5.00	100.00	27.82	7.73	71.70	5.00	5.00	100.00	6.97	6.97	S
TKPP1-03	85.00	18.70	18.30	100.00	22.00	21.53	79.00	16.00	12.00	100.00	20.25	15.19	S
US-1	85.00	5.00	5.00	100.00	5.88	5.88	86.70	5.00	5.00	100.00	5.77	5.77	S
US-7	80.00	78.00	66.40	100.00	97.50	83.00	87.40	89.40	89.40	100.00	100.00	100.00	R
US-11	77.70	75.30	65.70	100.00	96.91	84.56	67.70	67.00	59.00	100.00	98.97	87.15	R

[a]98.1% technical grade of metalaxyl. 25% commercial grade of metalaxyl.

[b]Wilcoxon Signed Ranks Test at $P = 0.05$.

[c]Resistant (R) = both 5 and 100 μg/ml ≥ 40% growth at 0 μg/ml. Intermediate (I) = 5 μg/ml ≥ 40% growth of 0 μg/ml.

Sensitive (S) = both 5 and 100 μg/ml < 40% growth at 0 μg/ml.

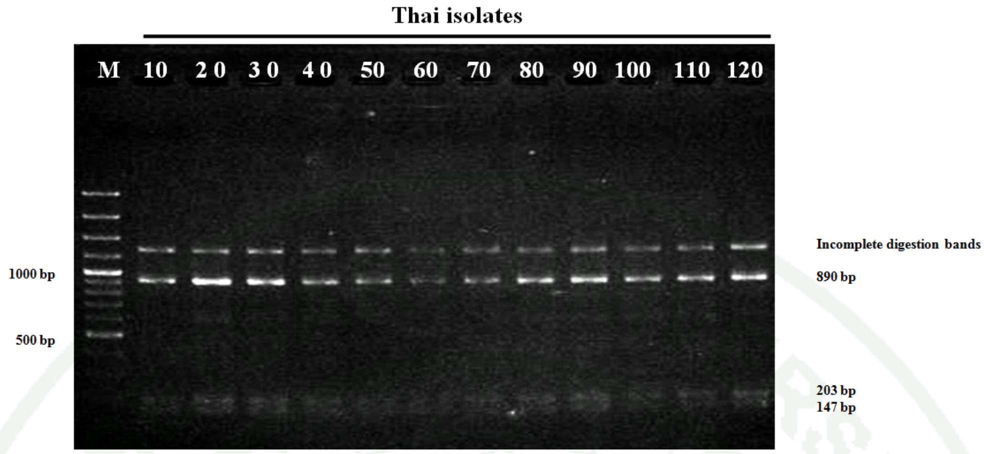

Figure 12 Restriction enzyme digestions of PCR products amplified from *Phytophthora infestans* Thai isolates with primer pairs F2-R2 cut with *Msp*I. Lane marked M contains 100-bp ladders (Fermentas).

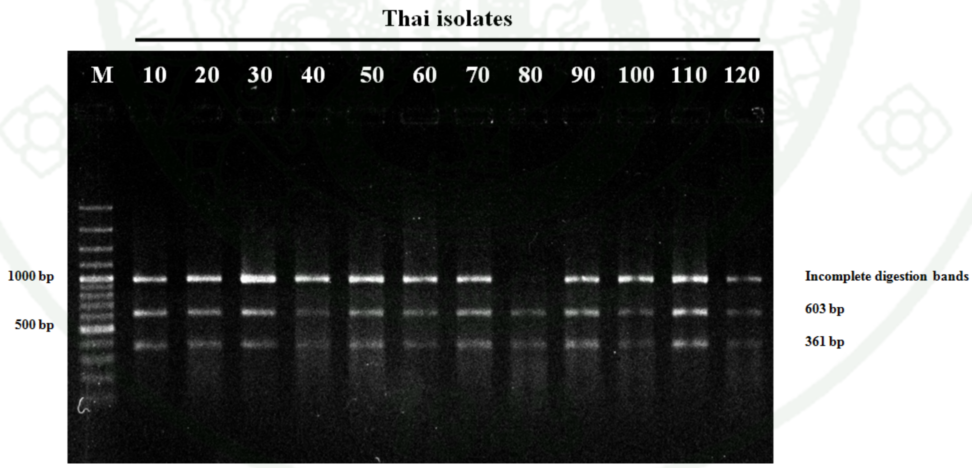

Figure 13 Restriction enzyme digestions of PCR products amplified from *Phytophthora infestans* Thai isolates with primer pairs F4-R4 cut with *Eco*RI. Lane marked M contains 100-bp ladders (Fermentas).

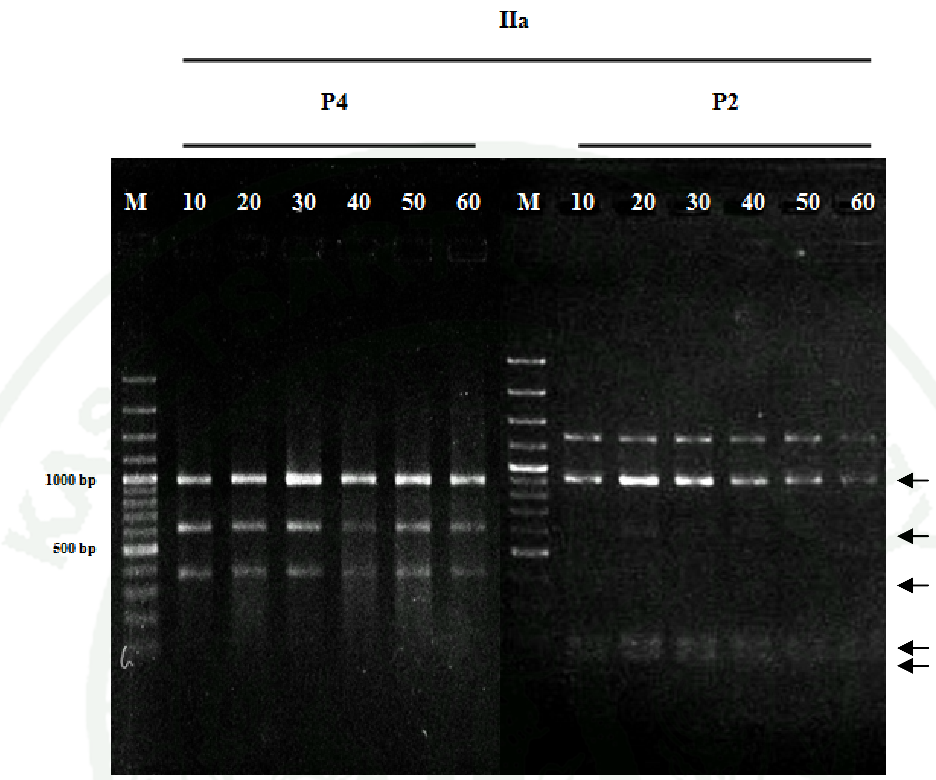

Figure 14 Restriction enzyme digestions of PCR products amplified from *Phytophthora infestans* Thai isolates with primer pairs F2-R2 and F4-R4 cut with *Msp*I and *Eco*RI, respectively. Amplifications were conducted with DNA from six isolates of mitochondrial DNA haplotype IIa. Lanes marked M contain 100-bp ladders (Fermentas). Arrows indicate specific amplified bands. The upper bands are incomplete digestion bands.

4. DNA Sequencing Variability

A total of 1,489 nucleotides in three portions of nuclear genes were sequenced corresponding to 348 nucleotides of Intron Ras, 600 nucleotides of Ras and 541 nucleotides of B-Tubulin (Table 34). Sequences of Intron Ras, Ras and B-Tubulin of *P. infestans* were obtained from Genbank (Table 35) and also used for analyses.

Table 34 Genes, genomic location and size among nuclear loci examined from *Phytophthora infestans*-Thai population

Fragment	Gene	Genomic location	Sequence (n[a])	Size of sequenced fragment (bp[b])	Size of unambiguously aligned region (bp[b])
Intron Ras	non-coding	nucleus	69	348	348
Ras	*PiYpt1*	nucleus	76	600	540
B-Tubulin	*β-tubulin*	nucleus	73	541	518

[a]n = number of isolates sequenced

[b]bp = base pair

Table 35 Genbank accession numbers for Intron Ras, Ras and B-Tubulin sequences used in this study

Fragment	GenBank accession number	Area collected
Intron Ras	U30474	USA
	GQ261027	Ecuador
Ras	U30474	USA
	EF367090, EF367094, EF367083	Costa Rica
	EF367077, EF367075	Mexico
	EF366974	Bolivia
	GU258154, GU258155	Colombia
B-Tubulin	AY564035, AY564036	Netherland
	EU079633, JF919545, JF919528, JF919533	USA
	GU258163, HQ639931	Colombia

4.1 Phylogenetic analysis based on the Intron Ras

Non coding regions on nuclear of *P. infestans* were analyzed by multiple alignments and phylogenetic analysis (Table 34-35). Multiple alignments of Intron Ras nucleotide sequences of 69 isolates, 60-Thai and 7-US, and 2 sequences from Genbank showed similar identity (Table 36).

A phylogenetic tree was constructed from the multiple alignments of the Intron Ras nucleotide sequences of 69 isolates (Fig. 15 and Appendix Table E32). The phylogenetic tree showed a similar degree of relationship between the *P. infestans* population.

4.2 Phylogenetic analysis based on the Ras

The Ras regions on nuclear of *P. infestans* were analyzed by multiple alignments and phylogenetic analysis (Table 34-35). Multiple alignments of Ras nucleotide sequences of 76 isolates, 60-Thai and 7-US, and 9 sequences from Genbank showed that the identities ranged from 99.6 to 100.0% (Table 37). No difference of Thai Ras nucleotide was showed among population (100.0%).

A phylogenetic tree was constructed from the multiple alignments of the Ras nucleotide sequences of 76 isolates (Fig. 16 and Appendix Table E32). The phylogenetic tree showed a similar degree of relationship among the *P. infestans*-Thai population (0% divergence). These Thai isolates had 0.2% difference nucleotides when its Ras compared with 12 6-3-18 ND, GU258155 and US-11 US980008.

4.3 Phylogenetic analysis based on the B-Tubulin

The B-Tubulin regions on nuclear of *P. infestans* were analyzed by multiple alignments and phylogenetic analysis (Table 34-35). Multiple alignments of B-Tubulin nucleotide sequences of 73 isolates, 58-Thai and 7-US, and 8 sequences from Genbank showed that the identities ranged from 99.6 to 100.0% (Table 38 and

Appendix Table E32). No difference of Thai B-Tubulin nucleotide was showed among population (100.0%).

A phylogenetic tree was constructed from the multiple alignments of the B-Tubulin nucleotide sequences of 73 isolates (Fig. 17). The phylogenetic tree showed a similar degree of relationship among the *P. infestans*-Thai population (0% divergence). These Thai isolates had 0.2% difference nucleotides when its B-Tubulin compared with AY564035, EU079633, GU258163, and JF919528 and 0.4% compared with AY564036, JF919533, JF919545, and HQ639931.

5. RAPD fingerprinting

Out of 42 primers screened, 17 generated reproducible amplification products from the isolates. The 17 oligonucleotide primers produced a total of 86 bands among the 45-Thai and 2-US *P. infestans* isolates (Table 39), and clearly scorable bands. Among them, 84 RAPD bands showed polymorphism (97.7%) (Table 39). Primer OPB 12 produced highest number of bands, 9. Fifteen primers gave the highest polymorphism (100%), except 2 primers; GAG CAC GGG G and GGG CGC GAG T, which gave 80%. The number of RAPD group for each primer is shown in Table 39. Primer OPC-02 gave the highest number of RAPD genotypes, 12 genotypes.

Figure 18 shows a dendrogram of the relations among Thai and US isolates as revealed by RAPD. The similarity coefficient between the 47 isolates of *P. infestans* are in the range of 0.54 - 0.97. The 17 RAPD primers could separate the population into 2 big groups. The differentiation in these genotypes was not correlated with any phenotypic characters such as mating type, metalaxyl sensitivity, mtDNA haplotypes, sizes of sporangia and oospore, colonial characteristics on rye A agar and potato tuber slices, and lesion sizes on potato leaflets cv. Atlantic and Spunta.

Table 36 Percent identities (top right) and differences (bottom left) among the Intron Ras nucleotide sequences

		Percent Identity										Isolate
		1	2	3	4	5	6	7	8	9	10	
Divergence	1		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	U30474
	2	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	GQ261027
	3	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	Thai isolates
	4	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	12 6-3-18 ND
	5	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	US-1 US940501
	6	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	US-1 US94051
	7	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	US-11 US980008
	8	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	US-8 2125
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	US-8 3.7
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		US-8 BB

Table 37 Percent identities (top right) and differences (bottom left) among the Ras nucleotide sequences

		Percent Identity																	Isolate
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Divergence	1		99.8	100.0	100.0	100.0	99.8	100.0	100.0	100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	U30474
	2	0.2		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	12 6-3-18 ND
	3	0.0	0.2		100.0	100.0	99.8	100.0	100.0	100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	Thai isolates
	4	0.0	0.2	0.0		100.0	99.8	100.0	100.0	100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	US-1 US940501
	5	0.0	0.2	0.0	0.0		99.8	100.0	100.0	100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	US-1 US94051
	6	0.2	0.2	0.0	0.0	0.0		99.8	99.8	99.8	99.6	99.8	99.8	99.8	99.8	99.8	99.8	99.8	US-11 US980008
	7	0.0	0.2	0.0	0.0	0.0	0.2		100.0	100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	US-8 2125
	8	0.0	0.2	0.0	0.0	0.0	0.2	0.0		100.0	99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	US-8 3.7
	9	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0		99.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	US-8 BB
	10	0.2	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.2		99.8	99.8	99.8	99.8	99.8	99.8	99.8	GU258155
	11	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2		100.0	100.0	100.0	100.0	100.0	100.0	GU258154
	12	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0		100.0	100.0	100.0	100.0	100.0	EF367094
	13	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0		100.0	100.0	100.0	100.0	EF366974
	14	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0		100.0	100.0	100.0	EF367075
	15	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0		100.0	100.0	EF367077
	16	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0		100.0	EF367083
	17	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0		EF367090

Table 38 Percent identities (top right) and differences (bottom left) among the B-Tubulin nucleotide sequences

		Percent Identity																Isolate
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Divergence	1		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.6	99.8	99.6	99.6	99.6	99.6	99.8	12 6-3-18 ND
	2	0.2		100.0	100.0	100.0	100.0	100.0	100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	Thai isolates
	3	0.2	0.0		100.0	100.0	100.0	100.0	100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-1 US940501
	4	0.2	0.0	0.0		100.0	100.0	100.0	100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-1 US94051
	5	0.2	0.0	0.0	0.0		100.0	100.0	100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-11 US980008
	6	0.2	0.0	0.0	0.0	0.0		100.0	100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-8 2125
	7	0.2	0.0	0.0	0.0	0.0	0.0		100.0	99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-8 3.7
	8	0.2	0.0	0.0	0.0	0.0	0.0	0.0		99.6	99.8	99.6	99.8	99.8	99.6	99.8	99.6	US-8 BB
	9	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4		99.8	100.0	99.8	99.8	99.8	99.8	100.0	JF919545
	10	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2		99.8	100.0	100.0	99.8	100.0	99.8	AY564035
	11	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.0	0.2		99.8	99.8	99.8	99.8	100.0	AY564036
	12	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2		100.0	99.8	100.0	99.8	EU079633
	13	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2	0.0		99.8	100.0	99.8	GU258163
	14	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2		99.8	99.8	HQ639931
	15	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.2	0.0	0.2	0.0	0.0		99.8	JF919528
	16	0.2	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.0	0.2	0.0	0.2	0.2	0.2	0.2		JF919533

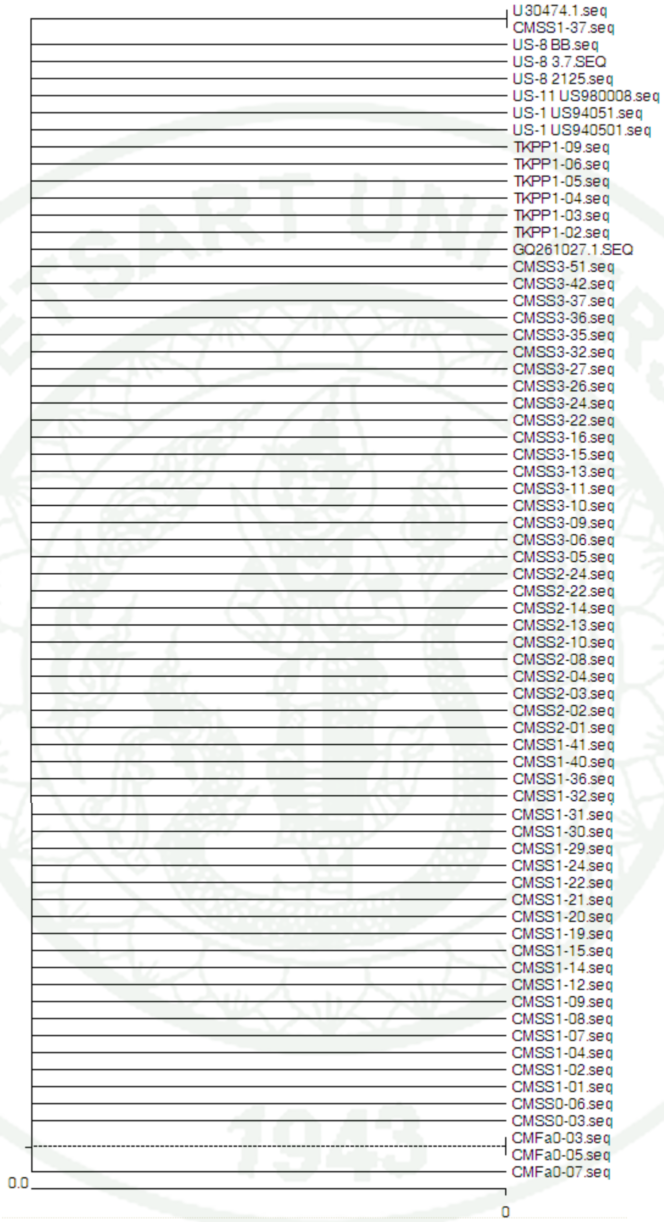

Figure 15 Phylogenetic tree of Intron Ras sequences among *Phytophthora infestans* population from Thailand (beginning with CM and TK), the United State (beginning with US and number) and Genbank (beginning with U3 and GQ). Scale bar is shown at the bottom of dendrogram.

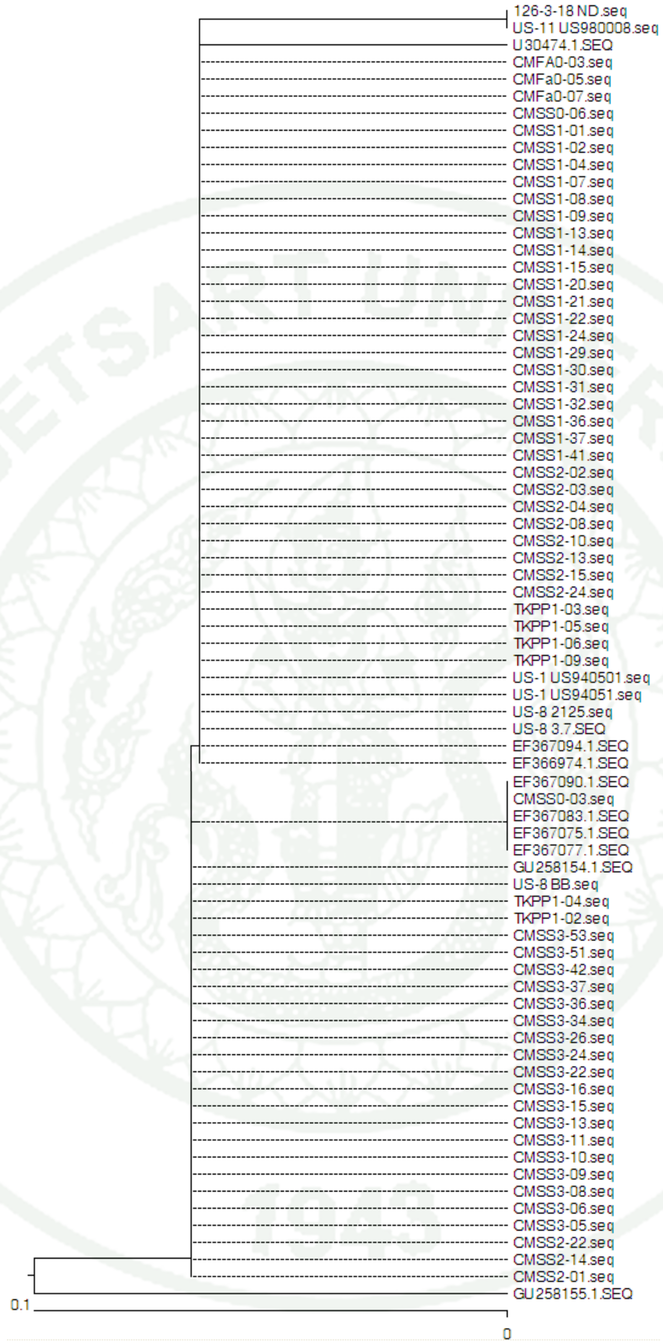

Figure 16 Phylogenetic tree of Ras sequences among *Phytophthora infestans* population from Thailand (beginning with CM and TK), the United State (beginning with US and number) and Genbank (beginning with EF, GU, and U3). Scale bar is shown at the bottom of dendrogram.

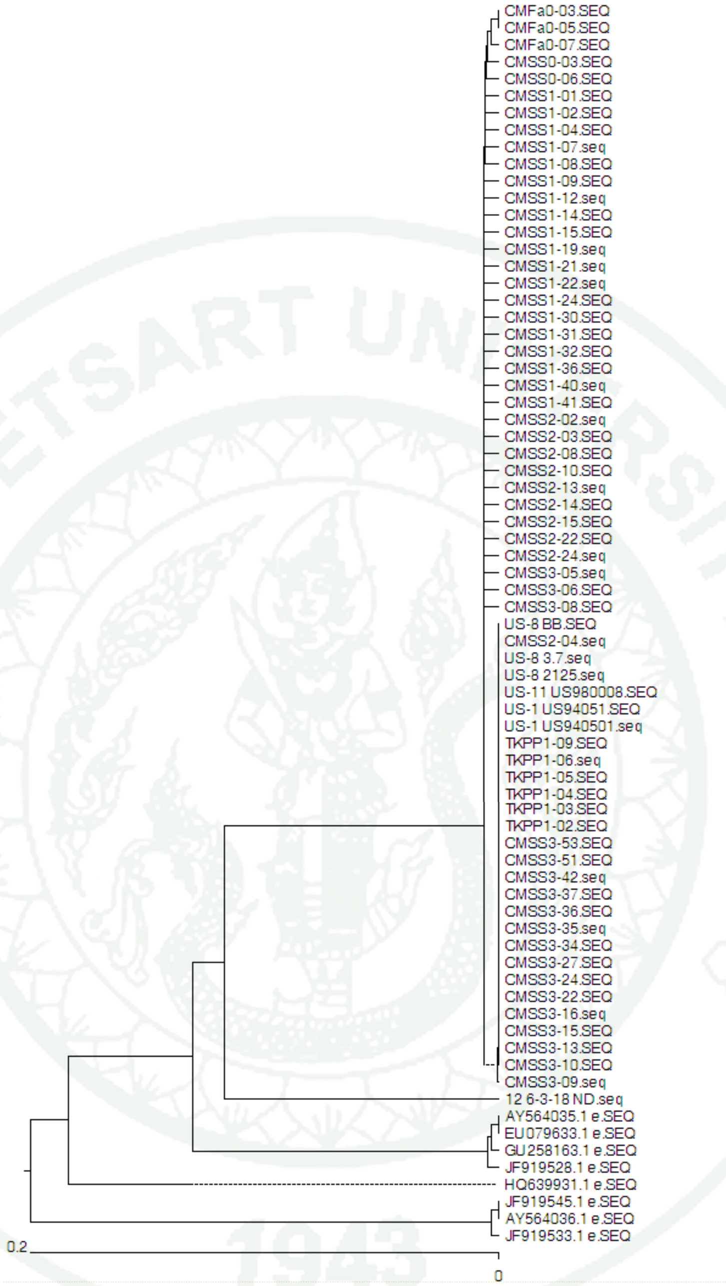

Figure 17 Phylogenetic tree of B-Tubulin sequences among *Phytophthora infestans* population from Thailand (beginning with CM and TK), the United State (beginning with US and number) and Genbank (beginning with AY, EU, GU, HQ and JF). Scale bar is shown at the bottom of dendrogram.

Table 39 Polymorphisims provided by the RAPD primers within *Phytophthora infestans* populations

Sequence (5'-3')	RAPD primer	Number of scorable PCR products	Number of polymorphic PCR products	Percentage of polymorphic bands	Number of RAPD genotypes
GGC TGC GAC A	OPY-5	6	6	100.0	9
AGA GCC GTC A	OPY-7	6	6	100.0	7
CCA GAT GCA C	OPE-3	6	6	100.0	5
GGT GAC GCA G	OPB-07	4	4	100.0	5
CCT TGA CGC A	OPB-12	9	9	100.0	13
AGG GAA CGA G	OPB-17	7	7	100.0	10
GTG AGG CGT C	OPC-02	6	6	100.0	12
ACG ACC GAC A	BA97	6	6	100.0	10
AGT CGG GTG G	S11	5	5	100.0	5
CCT GGG TGG A	-	1	1	100.0	2
CCG GCC CCA A	-	2	2	100.0	3
GTC CCA GAG C	-	5	5	100.0	11
GGG CAC GCG A	-	4	4	100.0	6
GAG GGC GAG G	-	4	4	100.0	5
GAG CAC CAG G	-	5	5	100.0	9
GAG CAC GGG G	-	5	4	80.0	5
GGG CGC GAG T	-	5	4	80.0	6
Total		86	84	97.7	-

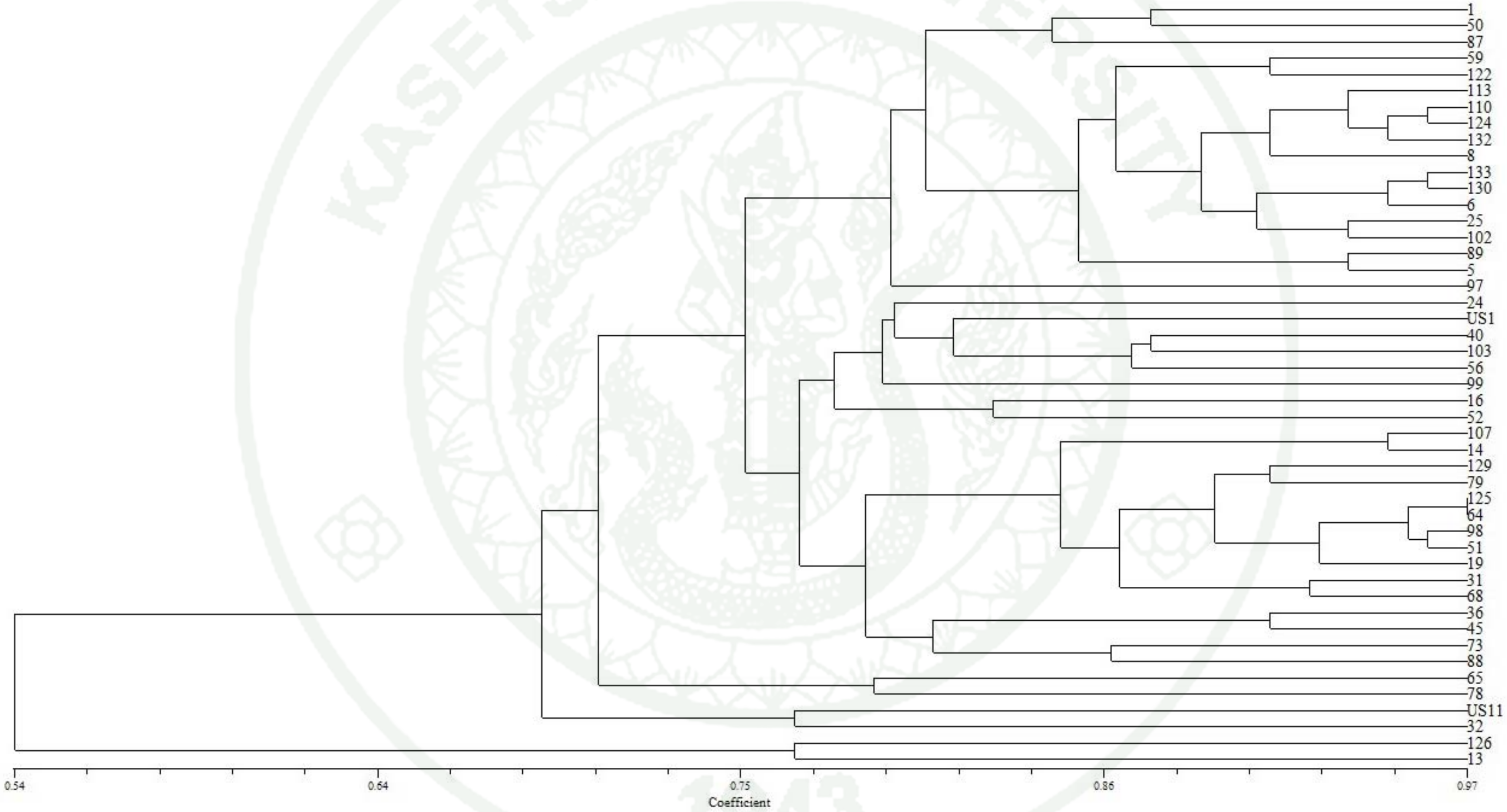

Figure 18 Dendogram of genetic relationships based on genetic similarity between 47 isolates of *Phytophthora infestans* using RAPD

DISCUSSION

Culture media for *Phytophthora infestans*

The main aim of this study was to investigate substrates used in Thailand and Asian countries for preparation *P. infestans* culture media. Generally, common culture media of this fungus are prepared from rye grain and V8 juice. In Thailand and Asian countries, rye grain is not available or difficult to obtain. Furthermore, at least 4 isolates of the Thai-isolates collection did not grow on V8 agar. In this study, culture media prepared from various substrates were thick and not completely dissolved; RKB, SFW, BSS, BB, SB, MB, WB and AB, but gave better growth than clear media (completely dissolved); SG, CM, JT, dried maize, corn husk and cob and unhusked rice. Isolates grown on RKB, SFW, BSS and BB media exhibited good mycelial growth, and abundant production of limoniform and semipapillate sporangia typical of *P. infestans* (Erwin and Ribeiro, 1996). Although SFW medium was a good medium, it needed vigorous mix than other media before pouring. If this medium was not thoroughly mixed prior to pouring, growth was poor. Various organic media stimulate growth and sporulation of *Phytophthora* and *Pythium* (Erwin and Ribeiro, 1996). Plant sterols in pea seed (Elliott, 1977), soybean (Marshall *et al*., 2001) and rye and oat (Skidmore *et al*., 1984) have growth stimulating properties. RKB, SFW, BSS and BB media containing β-sitosterol induced sporulation of few-sporulating isolates; US-1, US-7 and US-11. Medina and Platt (1999) successfully used clarified V8 juice containing β-sitosterol as a medium for sporulation. Moreover, BB and RKB supplemented with β-sitosterol enhanced oospore production of *P. infestans*. Medina and Platt (1999) reported that carrot and V8 juice agar with β-sitosterol enhanced oospore production.

Some Thai-isolates showed no growth on V8 agar. In this study, BB, BSS, and RKB media supported growth of all Thai-isolates. Therefore, these media can be substituted for V8 agar and rye A agar which were a common media for this pathogen. However, Thai and US isolates responded differently to those selected media in terms of visible aerial mycelium and radial growth. The colonies of US isolates were much thinner than Thai isolates indicating that nutrient utilization by each isolate might depend on its genotype. Peters *et al*. (1998) noted that the viability of cultures of *P. infestans* can still be maintained for at least a year and possibly for much longer depending upon the genotype.

The selected media were used for other applications such as long term storage. BB, BSS, SFW and RKB media could be used to maintain viability and asexual reproduction for 8 months. Isolates were successfully stored on these media up to 8 months. Various media have been evaluated for long-term storage of *P. infestans* using either agar-based media or media using whole seed prepared from ingredients such as corn or rye seed (Goth 1981; Hodgson and Grainger, 1964; Snieszko *et al*., 1947). Peters *et al*. (1998) reported the best media for maintaining the viability of *P. infestans* were media using corn and rye. Goth (1981) reported that media using sweet corn seed could be used to maintain for 12 months. In our experiment, agar-based medium made from sweet corn successfully supported both growth and sporulation of Thai and US isolates but not long-term storage.

The price of each substrate used in this study was compared and indicated that the local substrates was cheaper than imported substrate such as rye grains and those substrates were available in both local market and department store (Appendix Table E33).

The culture media prepared from locally available black bean, red kidney bean, and black sesame seeds using a protocol described for the preparation of rye A agar were effective in studies on *P. infestans.*

Morphological characteristics of *Phytophthora infestans*-Thai-isolates

These studies demonstrate the variation in morphologies within the population of *P. infestans* Thai isolates collected from Chiang Mai and Tak provinces.

In this study, all Thai isolates were showed the good growth at 18-20^{o}C on rye A, rye B, or modified media. Even, the maximum temperature for *P. infestans* for growth is 26^{o}C (Erwin and Ribeiro, 1996). Incubation temperature at 23^{o}C or higher trended to slow their growth.

Sporangia, pedicel and oospore of the isolates were divided into 8 groups depending on their variable size. Although, this study found association between size of reproductive propagules (pedicel and oospore) and source of isolates, no association between size of reproductive propagules and aggressiveness on potato leaflets. Hüberli (2001) found no significant relationship between radial growth rates and pathogenicity of the 73 *P. cinnamomi* isolates. Hantula *et al*. (2000) studied on the morphological measurements of oogonia and oospore among *P. cactorum* isolates found some differences in size. However, size of oogonia and oospore were not sufficient for the identification of genetic group or host specificity of the isolates. In contrast to Linde *et al.* (1999) who found a significant correlation between lesion length and growth of *P. cinnamomi* on agar.

After hyphal tip culture of *P. infestans* isolates, they were inoculated onto agar media and potato tuber slices to investigate the features of colonial growths. The cottony, powdery and concentric ring types of colonial growths were expressed on rye A agar. The sporangial and mycelial types of colonial growths were expressed on potato tuber slices. The isolates showed various growth characters on V8 agar. There was no specific feature or cluster associated with aggressiveness on potato leaflets. Kato *et al*. (1992) found that isolates produced aporangia on potato tuber slice also grew well on V8 agar but not on oatmeal agar were mating type A1. Isolates produced only mycelia and less grew on V8 agar but grew well on oatmeal agar were mating type A2.

The variations of the isolates based morphological characteristics were revealed in this study. However, its characteristic may be changed or unstable because the temperatures during culture or components of culture media (Zentmyer *et al.*, 1979; Sujkowski, 1986; Erwin and Ribeiro, 1996; Cooke and Lees, 2004).

Pathogenicity and aggressiveness of *Phytophthora infestans* isolates

The pathogenicity and aggressiveness of all 132 Thai-isolates were investigated on potato (cv. Atlantic and Spunta) and tomato (cv. Delta and Seeda) leaflets. Three parameters; LA, IF and SC, of aggressiveness were recorded on both hosts. One representative isolate, isolate 39 was randomly selected from the largest lesion producing isolates on both potato leaflets cv. Atlantic and Spunta, for host range test by inoculating on ornamental plants and weeds belonging to the family Solanaceae.

All isolates collected from infected potato leaflets infected both on potato and tomato leaflets and produced sporangia on the lesion, however the less sporangia were observed in some isolates. Mukalazi *et al*. (2001) reported that the 81 *P. infestans*-Ugandan isolates were pathogenic on potato but only 44.2% were pathogenic on detached tomato leaves. Lebreton *et al*. (1999) also found different in its pathogenicity on potato and tomato. Fontem *et al*. (2005) reported that the pathogenicity of *P. infestans* obtained from potato, tomato and huckleberry infected their primary host as well as the other two plant hosts. Chen *et al*. (2008 and 2009) reported that tomato isolates before 1997 were aggressive to tomato but not potato, most isolates obtained after 1998 were aggressive both on potato and tomato.

The lesion size on all host were compared resulting that tomato leaflets cv. Seeda had significantly smaller than another hosts whereas potato leaflet cv. Atlantic showed the largest. This finding reveals that *P. infestans* isolates from potato were more aggressive on potato leaflets. The results of pathogenicity test from Fontem *et al*. (2005) showed that the isolates from huckleberry were more aggressive on potato and tomato than on huckleberry, the potato isolates were the most aggressive on potato and least on huckleberry, and the tomato isolates were equally aggressive on potato and tomato, and significantly less on huckleberry. Legard *et al*. (1995) and Mukalazi *et al*. (2001) also reported similar finding. Erselius *et al*. (1998) concluded that the isolates attacking potato and tomato in Kenya and Uganda were different from each other by the analysis on RFLP fingerprint, Gpi genotype, aggressiveness on the potato and tomato, and isolation characteristics. Specialization of *P. infestans* on potato and tomato had been detected at other sites (Fry *et al*., 1991; Koh *et al*., 1994; Oyarzun *et al*., 1998; Suassuna *et al*., 2004).

The aggressiveness of the population was unassociated with the source of isolates, size of reproductive propagules, and metalaxyl sensitivities of isolates. Fontem *et al*. (2005) reported that the aggressiveness of the isolates was increased with their corresponding resistance to metalaxyl. Kadish and Cohen (1998) found that metalaxyl resistant isolates produced larger lesion in potato leaflets than metalaxyl sensitive isolates, but no singnificant differences were recorded in the infection frequency or sporulation capacity. Pliakhnevich and Ivaniuk reported that the aggressiveness of Belarusian *P. infestans* isolates varied greatly but differed insignificantly between the regions of collection.

The many cultivated solanaceous plant and weeds were not an alternative hosts in Thailand because symptom could not be observed (Table 30) except eggplant, chilli pepper and petunia leaves. However, the natural infection of *P. infestans* on the alternative host has not been found and reported in Thailand.

The cultivated solanaceous crops have been reported as source of inoculums of late blight such as petunia leaves (Peterson, 1947; Platt, 1999), eggplant (Birch and Whisson, 2001), and tobacco (*N. acuminate* and *N. clevelandii*) (Vartanian and Endo, 1985). Weed also plays a role as alternative host of *P. infestans* such as ground-cherry (Peterson, 1947), tomatillo (Erwin and Ribeiro, 1996), jimson weed (Vartanian and Endo, 1985), climbing nightshade (Platt, 1999), hairy nightshade (Punja *et al*., 1998). Also list as host are the non-solanaceous plants such as morning glory, common morning glory, napalese smart weed and sowthistle (Erwin and Ribeiro, 1996; Raj *et al*., 1976).

However, changes in pathogenicity of *P. infestans* are known to occur and have been perceived after continued culture on artificial media (Gallegly, 1968) or resistant varieties of potato (Graham *et al*., 1961).

Genetic distribution and characteristics of *Phytophthora infestans*-Thai isolates

The phenotypic and genotypic characteristics of Thai isolates were characterized by using mating type, metalaxyl sensitivity test and mtDNA haplotype.

Ninety one and nine percent of 132 Thai isolates were intermediate and sensitive to metalaxyl, respectively. No resistant isolates were found among Thai population. The association between metalaxyl sensitivity of the isolates and sources of the isolates where a phenylamide fungicide had been applied was found. It is indicated that all locations, except Long Khot, were regularly applied with phenylamide fungicide affecting on fungicide resistance of the isolates. All 44 Thai-isolates collecting in 1994 from ChiangMai were sensitive to metalaxyl (Gotoh *et al*., 2005). Jaimasit and Prakop (2010) found 2 of 15 isolates collecting from Sansai (ChiangMai province) and 9 of 21 isolates collecting from Phobpra (Tak province) were resistant to metalaxyl. The resistance to metalaxyl was first recorded in Ireland and the Netherlands in 1980 (Davidse *et al*., 1981; Dowley and O'Sullivan, 1981), relatively after its introduction in 1977 (Schwinn *et al.*, 1977; Urech *et al.*, 1977). The change in sensitivity to metalaxyl of *P. infestans* was reported at other sites (Cooke *et al*., 2006; Lehtinen *et al*., 2007; Mayton *et al*., 2000; McLeod *et al*., 2001; Reis *et al*., 2003; Tombolato, 2002). These studies found that the levels of metalaxyl sensitivities of population were not associated with size of reproductive propagules.

The “old” population was represented by the A1 mating type and Ib mtDNA haplotype, whereas the “new” population consisted of the isolates of both A1 and A2 mating types and Ia and IIa mtDNA haplotypes (Statsyuk *et al*., 2010). In this study, all isolates were mating type A1 and mtDNA haplotype IIa. This indicated that *P. infestans* Thai-isolates was the “new” population and had the unique characteristic by using these two markers. In 1994, only one document referred that Thai isolates were mating type A1 and A2 with ratio 50:50. This mating type A2 isolates contained mtDNA haplotype Ia. All isolates showed resistance to metalaxyl (Gotoh *et al*., 2005). However, the other finding by Sanyong *et al*. (1993) and Jaimasit and Prakob (2010) showed all Thai isolates were mating type A1 and IIa for mtDNA haplotype. This finding, including this study, may indicated that mating type A2 isolate was not dominant or present in the natural field, and resulting in the unique mtDNA of Thai isolate. The mating type and mtDNA haplotype of *P. infestans* were reported at other parts of the world (Adler *et al*., 2004; Cooke *et al*., 2006; Dowley *et al*., 2000; Gotoh *et al*., 2005; Lehtinen *et al*., 2007; McLeod *et al*., 2001; Reis *et al*., 2003; Runno-Paurson *et al*., 2009; Shaw *et al*., 2007; Wangsomboondee *et al*., 2002).

Growth of filamentous fungi, including *Phytophthora*, takes place by cell wall expansion within a small region at the hyphal apex. A common feature of the cytoplasm at hyphal tips is the presence of large numbers of vesicles, among which different types may often be distinguished (Hemmes, 1983). It is believed that the vesicles are involved in the transport of materials required for secretion or cell wall synthesis from subapical regions of the cytoplasm to the region of the hyphal tip. Recent studies of vesicle transport and secretion have of vesicle transport and secretion have shown that related series of gene products, which include members of the Ras superfamily of monomeric GTP-binding proteins, are involved in these processes in mammals, yeast and *P. infestans* (Bennett and Scheller, 1993; Chen and Roxy, 1996; Novick and Brennwald, 1993). Analysis of *P. infestans*-genomic and cDNA sequences of this RAS containing *Piyptl* indicates that it contains five introns, one in the 5'-untranslated region (Chen and Roxy, 1996).

Cellular microtubules are known to play an essential role in nuclear division as components of the mitotic spindle and dimeric tubulin. A key property of tubulin is its ability to assemble into microtubules via interaction between polymerized α-and *β*-tubulin monomers (heterodimer), and to undergo disassembly at appropriate times in the cell cycle. Microtubules are the site of action of an important family of agents that include fungicides (Keinath, 2007).

Recent molecular analysis has increased our understanding of the phylogenetic relationships, genetic variation and diversity, and population genetic (Goodwin, 1997; Cooke *et al*., 2000; Gomez-Alpizar *et al*., 2007). In this phylogenetic study is based on three sets of sequence data from nuclear gene; IntronRas, RAS and B-tubulin. The genetic variation among Thai population had no difference. Low genetic diversity of four nuclear loci; ITS, B-tubulin, Ras and Avr3a, as well as one mitochondrial gene; cytochrome c oxidase 1 (Cox1) was found within 80 sample of *P. infestans* isolates from solanaceous crops within several regions of Colombia and Venezuela (Cárdenas *et al*., 2011). In contrast, nuclear genetic variability was high in central Mexico resulting from sexual reproduction. After A2 mating type was introduced into Europe resulting in a shift from low to high nuclear genetic diversity in the Netherlands (Brurberg *et al*. 1999; Sujkowski *et al*., 1994; Zwankhuizen *et al*., 2000).

The genetics among Thai population were revealed by RAPD markers showing differentiation, but no correlation with the phenotypes. *Alternaria* spp. population isolated from infected grapefruit was genetically differentiated in terms of RAPD markers but unable to detect significant differences in pathogenicity between cultivars of grapefruit (Peever *et al.*, 2000). They speculated that the population was in the very early stages of differentiation on hosts and that any pathogenic differenced might have been too small to detect.

CONCLUSION

Biology, characteristics and variations of *P. infestans* isolates mainly collected from infected potato leaflets in northern Thailand, Chiang Mai and Tak provinces, were studied by using various criterions of morphology, capable of growth on media or substrates, phenotypic and genotypic markers, pathogenicity and aggressiveness and DNA sequencing.

P. infestans was unable to grow in general media using in mycological study except rye A agar which is used for culturing *P. infestans*. This study found at least 3 grains; black sesame, black bean and red kidney bean, which used for preparing the agar based media for culture and long-term storage. However, media made from those grains enhanced the growth and long-term storage of Thai isolates but not US isolates. Media made from fresh produce such as sweet corn supported the growth of Thai and US isolates but not for long-term storage.

All *P. infestans* Thai isolates produced typical lemon-shaped sporangia with showing mean of 40.66 μm length, 22.27 μm breadth and 1.84 L:B ratio. Oospore had a mean of 31.56 μm diameter. L:B ratio, pedicel, and oospore of the isolates were grouped by their size and then could be divided into 3 groups. It showed the variation in size of reproductive organs within population of *P. infestans*. Sizes of L:B ratio, pedicel and oospore had no associated with source of isolates. A total of 12 clusters of micro-morphology were classed by using multicharacter of micro-morphology. Colonial characters of 132-*P. infestans* Thai isolates on rye A agar showed cottony (26%), powdery (52%) and concentric ring (22%) types and on potato tuber slices cv. Atlantic showed mycelial (64%) and sporangial (36%) types. At least 3 Thai isolates had no growth on V8 agar. A total of 26 clusters of macro-morphology were classed by using multi-character of macro-morphology. Shannon index of micro-morphologies (1.13) revealing diversity of the isolates was less than macro-morphologies (2.53).

The pathogenicity and aggressiveness of 132 Thai isolates were investigated by inoculation on 2 cultivars of potato (cv. Atlantic and Spunta) and tomato (cv. Delta and Seeda) leaflets. It revealed that potato isolates infected on tomato leaflets as well as original host. Aggressiveness parameters such as infection frequency (IF), lesion area (LA), and spore capacity (SC), were recorded after inoculation on 2 cultivars of potato leaflets, potato tuber slices, and tomato leaflets. All isolates had 100 percent IF on both cultivars of potato tuber slices while 92, 72, 95 and 87 percent of isolates had the high percent IF on Atlantic, Spunta, Delta and Seeda leaflets, respectively. A total of 73, 55, 8, 2, 8 and 0 percent of isolates were grouped for the largest lesion-producing isolates on Atlantic leaflets, Spunta leaflets, Atlantic slices, Spunta slices, Delta leaflets and Seeda leaflets, respectively. A total of 2, 2, 1, 2, 1 and 1 percentage of the isolates were grouped for the highest sporulation-producing isolates on Atlantic leaflets, Spunta leaflets, Atlantic slices, Spunta slices, Delta leaflets and Seeda leaflets, respectively. The association was found between SC on Atlantic leaflets and source of isolates but not on the other host. The clusters of isolates were classed by using multicharacter of aggressiveness parameters on each host resulting that 8, 10, 9, 10, 9 and 7 clusters were classed for Atlantic leaflets, Spunta leaflets, Atlantic slices, Spunta slices, Delta leaflets and Seeda leaflets, respectively. Shannon index of the clusters of aggressiveness parameter revealing diversity of the isolates was 1.079, 1.443, 1.540, 1.544, 1.491 and 1.075 for Atlantic leaflets, Spunta leaflets, Atlantic slices, Spunta slices, Delta leaflets and Seeda leaflets, respectively. Atlantic leaflets had the largest LA compared with other hosts.

This is the first report in Thailand to reveal the pathogenicity on the alternative hosts; petunia, eggplant, and chilli pepper leaves, caused by aggressive-selected isolate.

All 132 isolates were characterized on metalaxyl sensitivity, mating type and mtDNA haplotype. Mating type A1 and IIa haplotype are dominated for the 132-Thai isolates. Ninety one percent of the isolates were intermediate to metalaxyl and 9 percent sensitive to metalaxyl.

Discrimination of *P. infestans* using global markers for *P. infestans* including metalaxyl sensitivity, mating type and mtDNA haplotype revealed that the unique characteristic was dominated in population. There were no resistant levels to metalaxyl in Thai isolates but more than 90 percent of isolates were intermediate which may be become to resistance in the future. Fungicide treatment, especially metalaxyl, in a potato field or potato tuber should be concerned to prevent fungicide resistance of the pathogen in Thailand. The information of mating type and mtDNA haplotype of Thai isolate can be used for exclusion the exotic isolate in plant quarantine step to prevent the genetic recombination which produces fungicidal resistant and aggressive isolates.

Phylogenetic analyses were performed on regions of nuclear gene sequences (IntronRas, RAS and B-tubulin) showing that *P. infestans*-Thai population had similar genetic level. The results obtained from different markers, especially genotypic markers, have indicated that the population of *P. infestans* in Thailand has the unique characteristic and not much on the genetic variation. RAPD fingerprinting of *P. infestans* Thai population using 17 primers showed variations on the genotypes.

Complete information of *P. infestans* genetic characteristic using the global markers is needed for worldwide to follow a change of this pathogen. For the future study of Thai isolates, RFLP fingerprinting with RG57 probe or allozyme analysis or SSR may be studied to fill a missing information which uses for a nomenclature, which is described for naming multilocus genotypes based on the International Organization for Standardization (ISO) two-letter country code and a unique number.

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APPENDICES

Appendix A

Isolation of *Phytophthora infestans*

Isolation of *Phytophthora infestans* from infected potato leaves (Fry, 2009)

1. An infected potato leaflet is incubated in moist chamber to induce sporangia in cool incubator at 18^{o}C for 24 hr.
2. Place spore-induced leaflet in a Petri dish which has 2-3 layers of moist filter paper.
3. A potato tuber is sterilized in 1% sodium hypochlorite for about 5 to 10 min and cut into slices that are 0.5 to 1 cm thick.
4. Cover a lesion with a potato slice in a Petri dish.
5. The mycelium will grow through the tuber slice. Check plates every 2-3 days for sporulation.
6. Brush a small piece of agar across the sporangia and place on agar plate. Up to 4 pieces of agar may be placed on one plate. Make several plates per sample.
7. After the pathogen is isolated on agar, it will transfer to rye A or rye B plates.

Appendix B

Media preparation

1. potato dextrose agar (PDA)

PDA is prepared by boiling 200 g of peeled and sliced potato tubers in distilled water until the potatoes are soft. Twenty grams of dextrose and 15 g of agar are added after the boiled potatoes are strained through the cheesecloth then adjusted to 1 liter before sterilizing in the autoclave at 121°C for 15 min, 15 Ib/in^2.

2. oat meal agar (OMA)

Seventy five grams of ground oats are boiled in 600 ml of distilled water at 45-55°C, added 20 g agar dissolved in 400 ml of water, and then sterilizing in the autoclave at 121°C for 15 min, 15 Ib/in^2.

3. pea agar (PA)

One hundred twenty grams of fresh pea pod are added to 800 ml of distilled water and sterilized at 121°C for 15 min, 15 Ib/in^2. The suspension is filtered. Twenty grams of glucose and 15 g of agar are added to the filtrate. The medium are adjusted to 1 liter volumes before sterilizing in the autoclave at 121°C for 15 min, 15 Ib/in^2.

4. 20% unclarified V8 agar (V8)

Two hundred milliliters of V8 juice are added to 800 ml of distilled water, along with 2 g of $CaCO_3$ and 0.05 g of *β*-sitosterol and mixed well with magnetic stirrer. Agar (15 g) is added and the suspension is then sterilizing in the autoclave at 121°C for 15 min, 15 Ib/in^2.

5. rye concentrate

Rye grains (600 g) are soaked in distilled water with an oxygen generator under dark conditions at room temperature (25°C) for 24 h. The liquid is retained and the swollen or germinated grains are ground in distilled water and incubated at 68°C for 1 h. The slurry is filtered through 3 layers of cheesecloth. The filtered liquid is combined with the retained liquid and used for preparing 2 liter of rye concentrate without sugar and agar. The rye concentrate is stored at -20°C until further use.

6. rye A agar (RA)

To prepare 5, 10, 15 and 20% rye A agar (RA), 50, 100, 150 and 200 ml of rye concentrate, respectively, are used for 1 liter of medium by adding 20 g and 15 g of glucose and agar, respectively, and then sterilize in the autoclave at 121°C for 15 min, 15 Ib/in^2.

7. rye B agar (RB)

Using the same protocol as preparation of rye A medium with the addition of 0.05 g/liter of β-sitosterol.

8. r-rye A (rRA)

A residual of rye grain from rye concentrate preparation is used for 5 liters of rRA medium with the same protocol used for pea agar (PA).

Appendix C

Metalaxyl preparation

1. Metalaxyl stock (100 mg/ml) is made by dissolving 1.20 g of 98.1% technical grade metalaxyl in 10 ml DMSO (Appendix Table C1).

Or

2. Metalaxyl stock (20 mg/ml) is made by dissolving 4 g of 25% commercial grade metalaxyl in 50 ml DMSO (Appendix Table C2).

3. Rye A agar was prepared. After autoclaving, allow the medium to cool down to about 50^{o}C and then add dimethylsulfoxide (DMSO) and/or metalaxyl stock as following table;

Appendix Table C1 Preparation of poisoned food from stock (100 mg/ml) of 98.1% technical grade metalaxyl

Solution	Amount (ml)		
	0 μg/ml	5 μg/ml	100 μg/ml
DMSO	1.00	0.95	0.00
Metalaxyl	0.00	0.05	1.00
Medium	995.00	995.00	995.00

Appendix Table C2 Preparation of poisoned food from Stock (20 mg/ml) of 25% commercial grade metalaxyl

Solution	Amount (ml)		
	0 μg/ml	5 μg/ml	100 μg/ml
DMSO	5.00	4.75	0.00
Metalaxyl	0.00	0.25	5.00
Medium	995.00	995.00	995.00

Appendix D

An Optimum of PCR Conditions

A maximum yield of the desired PCR product and reduce levels of nonspecific products were optimized by following steps:

1. A concentration of DNA template and an annealing temperature

The P2 primer was used for amplifying DNA template which re-suspended with sterile reverse osmosis water (RO-H_2O) obtained from total DNA extraction of *P. infestans* isolates.

1.1 Experiment

An amount of 0.00025, 0.0025, 0.025, 0.166, 0.25 and 0.625 μl of DNA templates (50 ng/ml quantified with a spectrophotometer) was added to a 25-μl master reaction mixture containing 1.5 μl of 10X PCR buffer (100 mM Tris-HCl, 500 mM KCl, and 0.8% Nonidet P40, pH 8.8), 1 μl of 25 mM $MgCl_2$, 2 μl of 2.5 mM dNTPs, 1 μl of 10 μM P2 forward primer, 1 μl of 10 μM P2 reverse primer, 0.125 μl of *Taq* polymerase (Fermentas), and then adjusted to final volume with sterile RO-H_2O.

Thermal cycling parameters were initial denaturation at 94^{o}C for 90 s, followed by 35 cycles consisting of denaturation at 94^{o}C for 40 s, annealing at 50.0, 54.3, 58.0, 59.3, 60.3, 62.7, 64.4, 65.2 and 68.1 for 60 s, and extension at 72^{o}C for 60 s. A final extension at 72^{o}C for 5 min followed.

Electrophoresis of amplified product was conducted on 1.8% agarose gels with TAE running buffer. The gel was run at 150 V for 45 min, and then stained with ethidium bromide. A 100-bp DNA ladder was included in each gel as a molecular size standard.

1.2 Result

The amounts of 0.00025, 0.0025, 0.025 and 0.25 μl of 50 ng/μl-DNA templates giving a concentration of 0.5, 5, 50 and 500 pg/μl were conducted by using

50.0, 54.3, 58.0 and 60.3°C of annealing temperature. The target fragment from primer P2 was amplified in length of 1,240 bp. A tendency of DNA was not amplified when the template DNA and the annealing temperature were reduced (Appendix Fig. D1). A strong concentration ranging from 5 to 500 pg/μl of DNA template gave two unexpected bands after PCR amplification.

The amounts of 0.025 and 0.25 μl of 50 ng/μl-DNA templates giving a concentration of 50 and 500 pg/μl were conducted by using 58.0, 59.3, 62.7, 64.4, 65.2 and 68.1°C of annealing temperature. The target fragment from primer P2 was amplified in length of 1,240 bp. The smeared backgrounds, non specific bands and primer dimer were occurred in lane 1 to 4 and 7 to 10 (Appendix Fig. D2). No DNA was amplified in lane 6 and 12 which was the DNA templates treated by 68.1°C of annealing temperature.

The amount of 0.166 μl of 50 ng/μl-DNA templates giving a concentration of 332 pg/μl was conducted by using 62°C of annealing temperature. The target fragment from primer P2 was amplified in length of 1,240 bp. The amplified DNA bands of samples were not clear or sharp (Appendix Fig. D3) which might was involved with the amount (1 μl) of primer used.

The amount of 0.625 μl of 50 ng/μl-DNA templates giving a concentration of 1,250 pg/μl was conducted by using 62°C of annealing temperature. The target fragment from primer P2 was amplified in length of 1,240 bp. The most of samples of DNA were sharp without backgrounds, non specific bands or primer dimer (Appendix Fig. D4) resulting from reducing the amount (0.33 μl) of primer.

1.3 Conclusion on optimum of DNA template and annealing temperature

Amount of 50 ng-DNA template optimized for PCR conditions was 0.625 μl (1,250 pg/μl) in 25-μl of final reaction. The optimum temperature of annealing for P2 primer was 62°C.

2. Sensitivity of two primer pairs

2.1 Primer P2

The P2 primer was used for amplifying DNA template which re-suspended with sterile reverse osmosis water (RO-H_2O), obtained from total DNA extraction of *P. infestans* isolates.

2.1.1 Experiment

An amount of 0.00025, 0.0025, 0.025, 0.25, 0.166 and 0.625 μl of DNA templates (50 ng quantified with a spectrophotometer) was added to a 25-μl master reaction mixture containing 1.5 μl of 10X PCR buffer (100 mM Tris-HCl, 500 mM KCl, and 0.8% Nonidet P40, pH 8.8), 1 μl of 25 mM $MgCl_2$, 2 μl of 2.5 mM dNTPs, 0.33 and 1 μl of 10 μM forward primer, 0.33 and 1 μl of 10 μM reverse primer, 0.125 μl of *Taq* polymerase (Fermentas), and then adjusted to final volume with sterile RO-H_2O. The final concentrations of forward primer and reverse primer in 25-μl PCR reaction were 0.132 and 0.4 pmole, respectively.

Thermal cycling parameters were initial denaturation at 94^{o}C for 90 s, followed by 35 cycles consisting of denaturation at 94^{o}C for 40 s, annealing at 62 for 60 s, and extension at 72^{o}C for 60 s. A final extension at 72^{o}C for 5 min followed.

Electrophoresis of amplified product was conducted on 1.8% agarose gels with TAE running buffer. The gel was run at 150 V for 45 min, and then stained with ethidium bromide. A 100-bp DNA ladder was included in each gel as a molecular size standard.

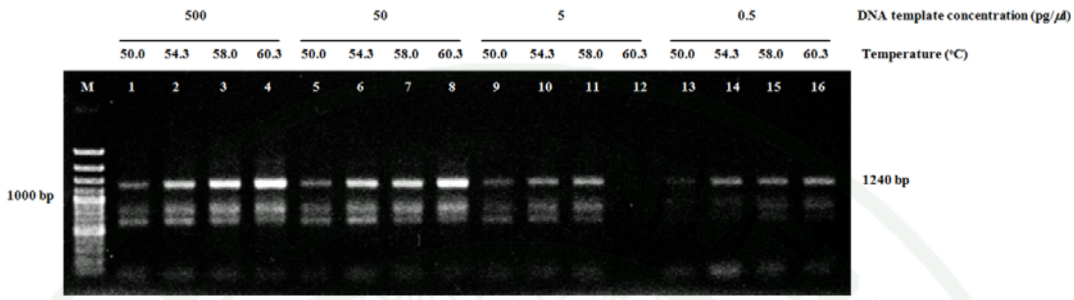
2.1.2 Result

The amounts of 0.00025, 0.0025, 0.025 and 0.25 μl of 50 ng/μl-DNA templates giving a concentration of 0.5, 5, 50 and 500 pg/μl were amplified with 1 μl of each 10 μM primer and conducted by using 50.0, 54.3, 58.0 and 60.3°C of annealing temperature (Appendix Fig. D1). The amounts of 0.025 and 0.25 μl of 50 ng/μl-DNA templates giving a concentration of 50 and 500 pg/μl were amplified with 1 μl of each 10 μM primer and conducted by using 58.0, 59.3, 62.7, 64.4, 65.2 and 68.1°C of annealing temperature (Appendix Fig. D2). The amount of 0.166 μl of 50 ng/μl-DNA templates giving a concentration of 332 pg/μl was amplified with 1 μl of each 10 μM primer and conducted by using 62°C of annealing temperature (Appendix Fig. D3). The target fragment from P2 primer pair (10 pmole in 25-μl PCR reaction) was amplified in length of 1,240 bp with unsatisfied. Therefore, the concentration of primer pair of P2 in 25-μl PCR reaction was optimized. The amount of 0.625 μl of 50 ng/μl-DNA templates giving a concentration of 1,250 pg/μl was amplified with 0.33 μl of each 10 μM primer and conducted by using 62°C of annealing temperature (Appendix Fig. D4). The most of samples of DNA were sharp without backgrounds, non specific bands or primer dimer because the sensitivities of primer pair were increased after reducing concentration to 0.132 pmole (Appendix Fig. D4).

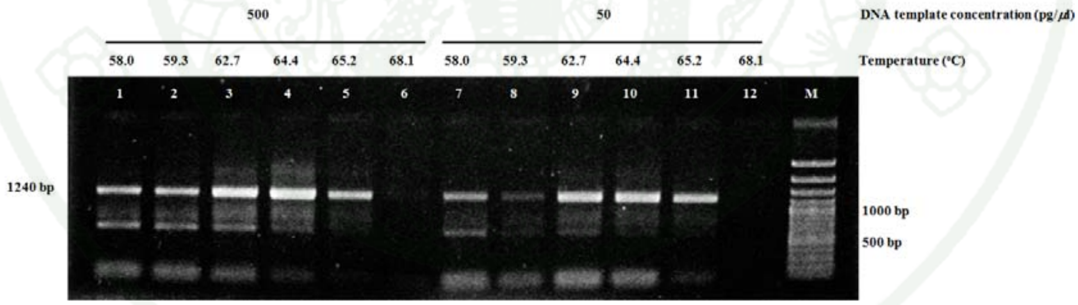
2.1.3 Conclusion on sensitivity of P2 primer pair

In 25-μl of final reaction, the optimum amount of 10 μM-P2 primer pair for PCR was 0.33 μl (final concentration was 0.132 pmole) at concentration of template DNA 1,250 pg/μl.

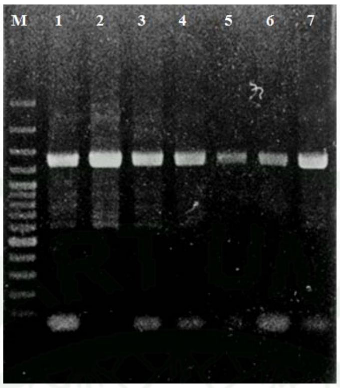
The P4 primer was used for amplifying DNA template which re-suspended with sterile reverse osmosis water (RO-H_2O), obtained from total DNA extraction of *P. infestans* isolates.



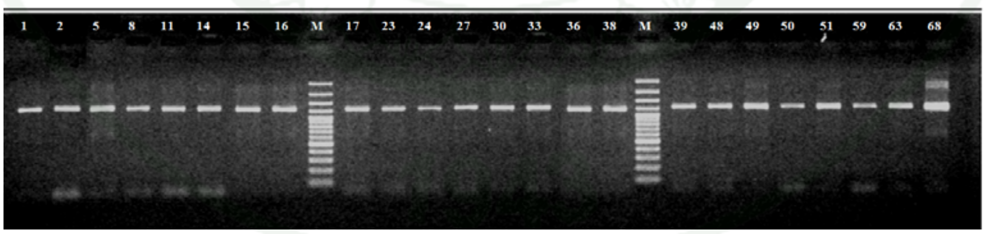
Appendix Figure D1 Amplification of DNA of *Phytophthora infestans* isolate 5 using 0.4 pmole-P2F and -P2R primers. DNA template at concentration of 0.5, 5, 50 and 500 pg/μl. Annealing temperature at 50.0, 54.3, 58.0 and 60.3^{o}C. Lanes marked M contain 100-bp ladders (Fermentas).



Appendix Figure D2 Amplification of DNA of *Phytophthora infestans* isolate 5 using 0.4 pmole-P2F and -P2R primers. DNA template at concentration of 50 and 500 pg/μl. Annealing temperature at 58.0, 59.3, 62.7, 64.4, 65.2 and 68.1^{o}C. Lanes marked M contain 100-bp ladders (Fermentas).



Appendix Figure D3 Amplification of DNA of *Phytophthora infestans* isolate 2, 6, 9, 10, 17, 28 and 29 in lane 1, 2, 3, 4, 5, 6 and 7, respectively, using 0.4 pmole-P2F and -P2R primers. DNA template at concentration of 332 pg/μl. Annealing temperature at 62^{o}C. Lanes marked M contain 100-bp ladders (Fermentas).



Appendix Figure D4 Amplification of DNA of 24-*Phytophthora infestans* isolates using 0.132 pmole-P2F and -P2R primers. DNA template at concentration of 1,250 pg/μl. Annealing temperature was at 62^{o}C. Lanes marked M contains 100-bp ladders (Fermentas).

2.2 Primer P4

2.2.1 Experiment

An amount of 0.625 μl of DNA templates (50 ng quantified with a spectrophotometer) was added to a 25-μl master reaction mixture containing 1.5 μl of 10X PCR buffer (100 mM Tris-HCl, 500 mM KCl, and 0.8% Nonidet P40, pH 8.8), 1 μl of 25 mM $MgCl_2$, 2 μl of 2.5 mM, the concentrations of P4 forward and reverse primers used in the experiment are listed in table below, 0.125 μl of *Taq* polymerase (Fermentas), and then adjusted to final volume with sterile RO-H_2O.

Appendix Table D1 Concentration of P4 primers used in the experiment

Condition	Original concentration (μM)	In 25-μl Reaction	
		Amount (μl)	Final concentration (pmol)
1	0.133	2, 3 , 4, 5, 6	0.01064, 0.01596, 0.02128, 0.0266, 0.03192
2	0.400	0.125, 0.25	0.002, 0.004
3	2.000	1, 2, 3, 4	0.08, 0.16, 0.24, 0.32
4	10.000	0.4, 0.6, 0.8, 1.0, 1.2	0.16, 0.24, 0.32, 0.4, 0.48

Thermal cycling parameters were initial denaturation at 94^{o}C for 90 s, followed by 35 cycles for the condition 1 and 4 or 40 cycles for the condition 2 and 3 (Appendix Table D1) consisting of denaturation at 94^{o}C for 40 s, annealing at 55 for 60 s, and extension at 72^{o}C for 60 s. A final extension at 72^{o}C for 5 min followed.

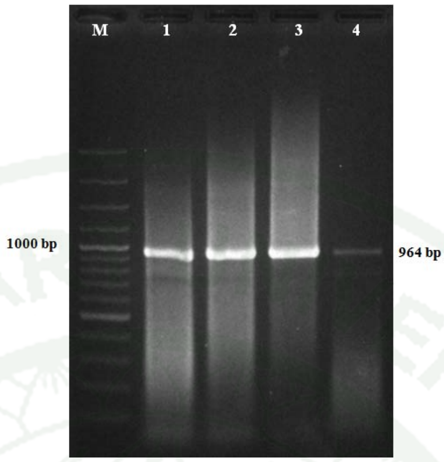
Electrophoresis of amplified product was conducted on 1.8% agarose gels with TAE running buffer. The gel was run at 150 V for 45 min, and then stained with ethidium bromide. A 100-bp DNA ladder was included in each gel as a molecular size standard.

2.2.2 Result

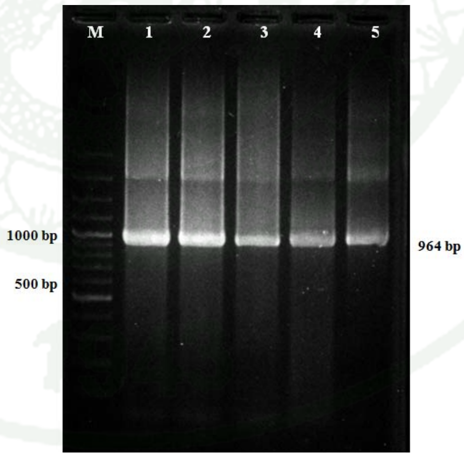
The amounts of 1, 2, 3 and 4 μl of each 2 μM-primer giving a concentration of 0.08, 0.16, 0.24 and 0.32 pmole were performed for 40 cycles. The DNA samples were amplified in thick bands for lane 1-3 with backgrounds. Although, the amplified fragment was found with no background in lane 4, the band was faint (Appendix Fig. D5). The amounts of 2, 3, 4, 5, and 6 μl of each 0.133 μM-primer giving a concentration of 0.01064, 0.01596, 0.02128, 0.0266 and 0.03192 pmole were performed for 40 cycles. The DNA samples were amplified in thick bands for all lanes with backgrounds and non specific bands (Appendix Fig. D6). The amounts of 0.125 and 0.25 μl of each 0.4 μM-primer giving a concentration of 0.002 and 0.004 pmole were performed for 40 cycles. The DNA samples were amplified in thick bands for all lanes with backgrounds and non specific bands (Appendix Fig. D7). The amounts of 0.4, 0.6, 0.8, 1.0, and 1.2 μl of each 0.4 μM-primer giving a concentration ranges from 0.16 to 0.48 pmole were performed for 35 cycles. The DNA samples were amplified in thick and sharp bands after amplifying at 55.3-60.3^{o}C with a dimer (Appendix Fig. D8).

2.2.3 Conclusion on sensitivity of P4 primer pair

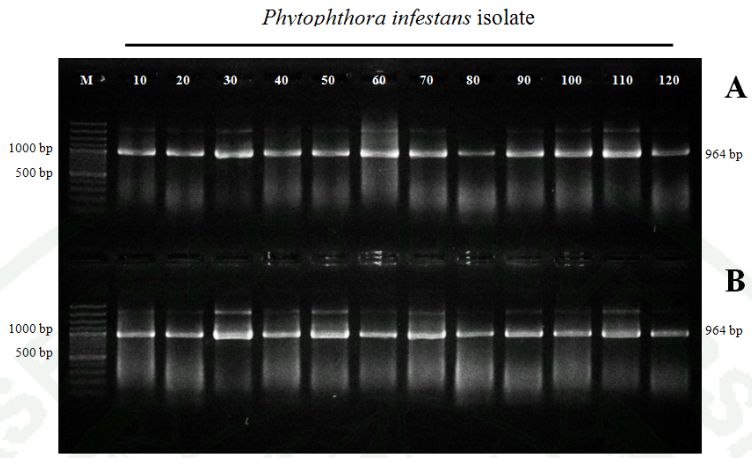
In 25-μl of final reaction, the optimum amount of 10 μM-P4 primer pair for PCR was 0.26 μl (final concentration was 0.104 pmole). Annealing temperature was at 55^{o}C.



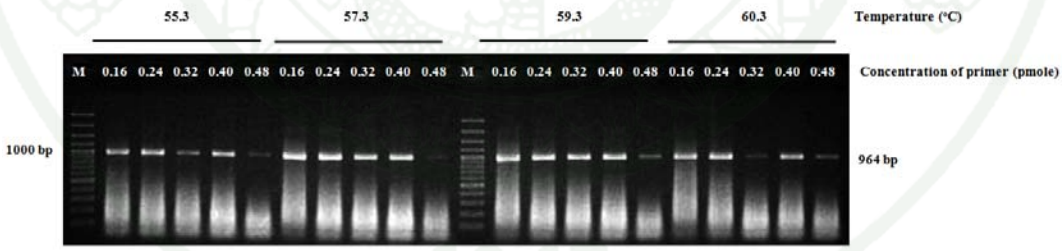
Appendix Figure D5 Amplification of DNA of *Phytophthora infestans* isolate 31 using lane 1: 0.32 pmole, lane 2: 0.24 pmole, lane 3: 0.16, and lane 4: 0.08 pmole-P4F and -P4R primers with 40 cycles. Lane marked M contains 100-bp ladders (Fermentas).



Appendix Figure D6 Amplification of DNA of *Phytophthora infestans* isolate 31 using lane 1: 0.32 pmole, lane 2: 0.24 pmole, lane 3: 0.16, and lane 4: 0.08 pmole-P4F and -P4R primers with 40 PCR-cycles. Lane marked M contains 100-bp ladders (Fermentas).



Appendix Figure D7 Amplification of DNA of *Phytophthora infestans* isolate 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, and 120 using A) 0.004 pmole and B) 0.002 pmole-P4F and -P4R with 40 PCR-cycles. Lanes marked M contain 100-bp ladders (Fermentas).



Appendix Figure D8 Amplification of DNA of *Phytophthora infestans* isolate 10 using 0.16, 0.24, 0.32, 0.40, and 0.48 pmole-P4F and -P4R with 35 PCR-cycles. Annealing temperature at 55.3, 57.3, 59.3 and 60.3^{o}C. Lanes marked M contain 100-bp ladders (Fermentas).

Appendix E

Table

Appendix Table E1 Source of *Phytophthora infestans* Thai isolates

Hyphal Tip Culture Code	Accession Number	Province	District	Sub-District	Month/Year
1	CMSS1-05	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
2	CMSS2-10	ChiangMai	SanSai	Mae FaekMai	Feb 2008
3	CMSS2-01	ChiangMai	SanSai	Mae FaekMai	Feb 2008
4	CMSS1-06	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
5	TKPP1-06	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
6	CMPh0-05	ChiangMai	Fang	-	Feb 2006
7	CMSS2-04	ChiangMai	SanSai	Mae FaekMai	Feb 2008
8	CMSS2-05	ChiangMai	SanSai	Mae FaekMai	Feb 2008
9	CMSS0-03	ChiangMai	SanSai	-	Feb 2006
10	CMPh0-03	ChiangMai	Fang	-	Feb 2006
11	CMSS1-07	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
13	CMSS3-16	ChiangMai	SanSai	Nong Han	Feb 2008
14	TKPP1-07	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
15	CMSS2-07	ChiangMai	SanSai	Mae FaekMai	Feb 2008
16	CMSS1-01	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
17	TKPP1-04	Tak	SanSai	Ruam Thai Phatthana	Dec 2007
18	CMSS3-08	ChiangMai	Phob Phra	Nong Han	Feb 2008
19	CMSS1-04	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
20	CMSS3-13	ChiangMai	SanSai	Nong Han	Feb 2008
21	CMSS2-03	ChiangMai	SanSai	Mae FaekMai	Feb 2008
22	CMSS2-15	ChiangMai	SanSai	Mae FaekMai	Feb 2008
23	CMSS2-08	ChiangMai	SanSai	Mae FaekMai	Feb 2008
24	CMSS1-08	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
25	CMSS3-15	ChiangMai	SanSai	Nong Han	Feb 2008
26	CMSS1-02	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
27	CMSS3-10	ChiangMai	SanSai	Nong Han	Feb 2008
28	CMSS0-06	ChiangMai	SanSai	-	Feb 2006
29	TKPP1-03	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
30	CMSS3-05	ChiangMai	SanSai	Nong Han	Feb 2008
31	CMSS2-02	ChiangMai	SanSai	Mae FaekMai	Feb 2008
32	CMSS3-09	ChiangMai	SanSai	Nong Han	Feb 2008
33	CMSS2-23	ChiangMai	SanSai	Mae FaekMai	Feb 2008
34	CMSS3-24	ChiangMai	SanSai	Nong Han	Feb 2008
35	CMSS3-26	ChiangMai	SanSai	Nong Han	Feb 2008
36	CMSS3-42	ChiangMai	SanSai	Nong Han	Feb 2008
37	CMSS2-24	ChiangMai	SanSai	Mae FaekMai	Feb 2008
38	CMSS1-31	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
39	CMSS1-21	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008

Appendix Table E1 (Continued)

Hyphal Tip Culture Code	Accession Number	Province	District	Sub-District	Month/Year
40	CMSS1-32	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
41	CMSS1-35	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
42	CMSS3-37	ChiangMai	SanSai	Nong Han	Feb 2008
43	CMSS1-19	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
44	CMSS3-27	ChiangMai	SanSai	Nong Han	Feb 2008
45	CMSS1-30	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
46	CMSS1-37	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
47	CMSS1-03	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
48	CMSS1-12	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
49	CMSS3-34	ChiangMai	SanSai	Nong Han	Feb 2008
50	CMSS1-16	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
51	CMSS3-36	ChiangMai	SanSai	Nong Han	Feb 2008
52	CMSS1-18	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
54	CMSS1-40	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
56	CMSS2-13	ChiangMai	SanSai	Mae FaekMai	Feb 2008
57	CMSS3-51	ChiangMai	SanSai	Nong Han	Feb 2008
58	CMSS3-54	ChiangMai	SanSai	Nong Han	Feb 2008
59	CMSS3-45	ChiangMai	SanSai	Nong Han	Feb 2008
60	CMSS3-44	ChiangMai	SanSai	Nong Han	Feb 2008
62	TKPP1-12	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
63	CMSS2-19	ChiangMai	SanSai	Mae FaekMai	Feb 2008
64	CMSS2-25	ChiangMai	SanSai	Mae FaekMai	Feb 2008
65	CMSS3-50	ChiangMai	SanSai	Nong Han	Feb 2008
66	CMSS2-20	ChiangMai	SanSai	Mae FaekMai	Feb 2008
67	CMSS1-33	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
68	CMSS3-03	ChiangMai	SanSai	Nong Han	Feb 2008
69	CMSS1-24	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
70	CMSS3-48	ChiangMai	SanSai	Nong Han	Feb 2008
72	CMSS3-06	ChiangMai	SanSai	Nong Han	Feb 2008
73	CMPh0-09	ChiangMai	Fang	-	Feb 2006
74	CMSS2-09	ChiangMai	SanSai	Mae FaekMai	Feb 2008
75	CMSS3-40	ChiangMai	SanSai	Nong Han	Feb 2008
76	TKPP1-02	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
77	CMSS1-15	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
78	CMSS2-22	ChiangMai	SanSai	Mae FaekMai	Feb 2008
79	CMSS3-28	ChiangMai	SanSai	Nong Han	Feb 2008
80	CMSS3-29	ChiangMai	SanSai	Nong Han	Feb 2008

Appendix Table E1 (Continued)

Hyphal Tip Culture Code	Accession Number	Province	District	Sub-District	Month/Year
81	CMSS3-31	ChiangMai	SanSai	Nong Han	Feb 2008
82	CMSS2-21	ChiangMai	SanSai	Mae FaekMai	Feb 2008
83	CMSS1-20	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
84	CMSS1-26	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
85	CMSS3-32	ChiangMai	SanSai	Nong Han	Feb 2008
86	CMSS1-29	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
87	CMSS0-05	ChiangMai	SanSai	-	Feb 2006
88	TKPP1-10	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
89	CMSS1-14	ChiangMai	SanSai	Chedi MaeKrua	Feb 2008
91	CMSS3-46	ChiangMai	SanSai	Nong Han	Feb 2008
92	CMSS3-52	ChiangMai	SanSai	Nong Han	Feb 2008
93	CMSS2-14	ChiangMai	SanSai	Mae FaekMai	Feb 2008
94	CMSS3-35	ChiangMai	SanSai	Nong Han	Feb 2008
99	TKPP1-05	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
100	CMSS3-47	ChiangMai	SanSai	Nong Han	Feb 2008
101	TKPP1-08	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
102	CMSS3-11	ChiangMai	SanSai	Nong Han	Feb 2008
103	CMSS2-18	ChiangMai	SanSai	Mae FaekMai	Feb 2008
104	CMPh0-07	ChiangMai	Fang	-	Feb 2006
105	TKPP1-09	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
106	CMSS3-01	ChiangMai	SanSai	Nong Han	Feb 2008
107	CMSS1-22	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
108	CMSS1-25	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
109	CMSS1-41	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
110	CMSS3-39	ChiangMai	SanSai	Nong Han	Feb 2008
111	CMSS3-38	ChiangMai	SanSai	Nong Han	Feb 2008
112	CMSS3-33	ChiangMai	SanSai	Nong Han	Feb 2008
113	CMSS3-22	ChiangMai	SanSai	Nong Han	Feb 2008
114	CMSS1-27	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
115	CMSS1-17	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
116	TKPP1-11	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
117	CMSS3-41	ChiangMai	SanSai	Nong Han	Feb 2008
118	CMSS3-23	ChiangMai	SanSai	Nong Han	Feb 2008
119	CMSS3-21	ChiangMai	SanSai	Nong Han	Feb 2008
120	CMSS3-14	ChiangMai	SanSai	Nong Han	Feb 2008
121	CMSS3-25	ChiangMai	SanSai	Nong Han	Feb 2008
122	CMSS3-49	ChiangMai	SanSai	Nong Han	Feb 2008

Appendix Table E1 (Continued)

Hyphal Tip Culture Code	Accession Number	Province	District	Sub-District	Month/Year
123	TKPP1-01	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
125	CMSS2-11	ChiangMai	SanSai	Mae FaekMai	Feb 2008
126	TKPP1-13	Tak	Phob Phra	Ruam Thai Phatthana	Dec 2007
127	CMSS1-39	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
128	CMSS1-42	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
129	CMSS3-20	ChiangMai	SanSai	Nong Han	Feb 2008
131	CMSS2-06	ChiangMai	SanSai	Mae FaekMai	Feb 2008
132	CMSS3-07	ChiangMai	SanSai	Nong Han	Feb 2008
133	CMSS1-28	ChiangMai	SanSai	Chedi MaeKrua	Jan 2008
134	CMSS3-04	ChiangMai	SanSai	Nong Han	Feb 2008
135	CMSS2-17	ChiangMai	SanSai	Mae FaekMai	Feb 2008
136	CMPr4-01	ChiangMai	Phrao	Long Khot	Feb 2009
137	CMPr4-02	ChiangMai	Phrao	Long Khot	Feb 2009
138	CMPr4-03	ChiangMai	Phrao	Long Khot	Feb 2009
139	CMPr4-04	ChiangMai	Phrao	Long Khot	Feb 2009
140	CMPr4-05	ChiangMai	Phrao	Long Khot	Feb 2009

Appendix Table E2 Chi-square tests for relation between the sources of isolates and the sizes of sporangia (L:B ratio)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.458(a)	12	.337
Likelihood Ratio	17.602	12	.128
Linear-by-Linear Association	.606	1	.436
N of Valid Cases	132		

a 15 cells (71.4%) have expected count less than 5. The minimum expected count is .18.

Appendix Table E3 Chi-square tests for relation between the sources of isolates and the sizes of pedicels

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.697(a)	12	.931
Likelihood Ratio	8.063	12	.780
Linear-by-Linear Association	.033	1	.856
N of Valid Cases	264		

a 11 cells (52.4%) have expected count less than 5. The minimum expected count is .57.

Appendix Table E4 Chi-square tests for relation between the sources of isolates and the sizes of oospores

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.827(a)	12	.156
Likelihood Ratio	17.752	12	.123
Linear-by-Linear Association	.789	1	.374
N of Valid Cases	264		

a 12 cells (57.1%) have expected count less than 5. The minimum expected count is .27.

Appendix Table E5 Chi-square tests for relation between the sources of isolates and the infection frequency on infected potato leaflets cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.378(a)	12	.832
Likelihood Ratio	7.621	12	.814
Linear-by-Linear Association	.295	1	.587
N of Valid Cases	132		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .02.

Appendix Table E6 Chi-square tests for relation between the sources of isolates and the infection frequency on infected potato leaflets cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.379(a)	12	.136
Likelihood Ratio	19.327	12	.081
Linear-by-Linear Association	1.342	1	.247
N of Valid Cases	132		

a 14 cells (66.7%) have expected count less than 5. The minimum expected count is .16.

Appendix Table E7 Chi-square tests for relation between the sources of isolates and the infection frequency on infected potato discs cv. Atlantic and Spunta

	Value
Pearson Chi-Square	.(a)
N of Valid Cases	131

a No statistics are computed because IF groups of potato disc cv. Atlantic and Spunta are constant.

Appendix Table E8 Chi-square tests for relation between the sources of isolates and the infection frequency on infected tomato leaflets cv. Delta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.901(a)	6	.821
Likelihood Ratio	3.961	6	.682
Linear-by-Linear Association	1.257	1	.262
N of Valid Cases	130		

a 10 cells (71.4%) have expected count less than 5. The minimum expected count is .12.

Appendix Table E9 Chi-square tests for relation between the sources of isolates and the infection frequency on infected tomato leaflets cv. Seeda

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.060(a)	12	.913
Likelihood Ratio	8.481	12	.747
Linear-by-Linear Association	1.725	1	.189
N of Valid Cases	130		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .03.

Appendix Table E10 Chi-square tests for relation between the sources of isolates and the lesion area on infected potato leaflets cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	22.151(a)	18	.225
Likelihood Ratio	21.340	18	.263
Linear-by-Linear Association	.826	1	.363
N of Valid Cases	132		

a 21 cells (75.0%) have expected count less than 5. The minimum expected count is .02.

Appendix Table E11 Chi-square tests for relation between the sources of isolates and the lesion area on infected potato leaflets cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24.854(a)	18	.129
Likelihood Ratio	27.406	18	.072
Linear-by-Linear Association	.182	1	.670
N of Valid Cases	132		

a 20 cells (71.4%) have expected count less than 5. The minimum expected count is .09.

Appendix Table E12 Chi-square tests for relation between the sources of isolates and the lesion area on infected potato tuber slices cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	25.877(a)	18	.103
Likelihood Ratio	28.707	18	.052
Linear-by-Linear Association	.520	1	.471
N of Valid Cases	131		

a 19 cells (67.9%) have expected count less than 5. The minimum expected count is .23.

Appendix Table E13 Chi-square tests for relation between the sources of isolates and the lesion area on infected potato tuber slices cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	21.746(a)	18	.243
Likelihood Ratio	16.486	18	.559
Linear-by-Linear Association	.186	1	.666
N of Valid Cases	131		

a 19 cells (67.9%) have expected count less than 5. The minimum expected count is .07.

Appendix Table E14 Chi-square tests for relation between the sources of isolates and the lesion area on infected tomato leaflets cv. Delta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.871(a)	18	.464
Likelihood Ratio	19.757	18	.347
Linear-by-Linear Association	1.935	1	.164
N of Valid Cases	130		

a 19 cells (67.9%) have expected count less than 5. The minimum expected count is .23.

Appendix Table E15 Chi-square tests for relation between the sources of isolates and the lesion area on infected tomato leaflets cv. Seeda

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.097(a)	12	.236
Likelihood Ratio	17.478	12	.132
Linear-by-Linear Association	2.874	1	.090
N of Valid Cases	130		

a 15 cells (71.4%) have expected count less than 5. The minimum expected count is .14.

Appendix Table E16 Chi-square tests for relation between the sources of isolates and the spore capacity on infected potato leaflets cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	32.036(a)	12	.001
Likelihood Ratio	19.292	12	.082
Linear-by-Linear Association	3.054	1	.081
N of Valid Cases	132		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .05.

Appendix Table E17 Chi-square tests for relation between the sources of isolates and the spore capacity on infected potato leaflets cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	98.889(a)	6	.000
Likelihood Ratio	26.655	6	.000
Linear-by-Linear Association	8.954	1	.003
N of Valid Cases	132		

a 10 cells (71.4%) have expected count less than 5. The minimum expected count is .09.

Appendix Table E18 Chi-square tests for relation between the sources of isolates and the spore capacity on infected potato tuber slices cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.555(a)	12	.971
Likelihood Ratio	5.539	12	.938
Linear-by-Linear Association	.003	1	.959
N of Valid Cases	131		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .02.

Appendix Table E19 Chi-square tests for relation between the sources of isolates and the spore capacity on infected potato tuber slices cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.641(a)	12	.647
Likelihood Ratio	11.151	12	.516
Linear-by-Linear Association	.039	1	.843
N of Valid Cases	131		

a 15 cells (71.4%) have expected count less than 5. The minimum expected count is .05.

Appendix Table E20 Chi-square tests for relation between the sources of isolates and the spore capacity on infected tomato leaflets cv. Delta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.219(a)	12	.768
Likelihood Ratio	8.221	12	.768
Linear-by-Linear Association	1.965	1	.161
N of Valid Cases	130		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .05.

Appendix Table E21 Chi-square tests for relation between the sources of isolates and the spore capacity on infected tomato leaflets cv. Seeda

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.740(a)	12	.966
Likelihood Ratio	4.889	12	.962
Linear-by-Linear Association	1.488	1	.223
N of Valid Cases	130		

a 17 cells (81.0%) have expected count less than 5. The minimum expected count is .02.

Appendix Table E22 Chi-square tests for relation between the sizes of micro-morphologies and the lesion area on infected potato leaflets cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	73.370(a)	77	.596
Likelihood Ratio	49.391	77	.994
Linear-by-Linear Association	.166	1	.683
N of Valid Cases	132		

a 93 cells (96.9%) have expected count less than 5. The minimum expected count is .01.

Appendix Table E23 Chi-square tests for relation between the sizes of micro-morphologies and the lesion area on infected potato leaflets cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	146.260(a)	99	.001
Likelihood Ratio	53.686	99	1.000
Linear-by-Linear Association	.468	1	.494
N of Valid Cases	132		

a 115 cells (95.8%) have expected count less than 5. The minimum expected count is .01.

Appendix Table E24 Chi-square tests for relation between the levels of metalaxyl sensitivity and the sources of isolates

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	72.674(a)	6	.000
Likelihood Ratio	49.510	6	.000
Linear-by-Linear Association	21.205	1	.000
N of Valid Cases	132		

[a]10 cells (71.4%) have expected count less than 5. The minimum expected count is .27.

Appendix Table E25 Chi-square tests for relation between the level of metalaxyl sensitivity and the sizes of sporangia (L:B ratio)

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	8.701(a)	2	.013
Likelihood Ratio	8.459	2	.015
Linear-by-Linear Association	7.184	1	.007
N of Valid Cases	132		

a 2 cells (33.3%) have expected count less than 5. The minimum expected count is .82.

Appendix Table E26 Chi-square tests for relation between the level of metalaxyl sensitivity and the sizes of pedicels

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.640(a)	2	.440
Likelihood Ratio	1.402	2	.496
Linear-by-Linear Association	.769	1	.381
N of Valid Cases	132		

a 3 cells (50.0%) have expected count less than 5. The minimum expected count is .09.

Appendix Table E27 Chi-square tests for relation between the level of metalaxyl sensitivity and the sizes of oospores

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.538(a)	2	.170
Likelihood Ratio	2.414	2	.299
Linear-by-Linear Association	.014	1	.905
N of Valid Cases	132		

a 4 cells (66.7%) have expected count less than 5. The minimum expected count is .27.

Appendix Table E28 Chi-square tests for relation between the metalaxyl sensitivities of isolates and the aggressive (lesion area) on infected potato leaflets cv. Atlantic

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.534(a)	3	. 534
Likelihood Ratio	.986	3	.805
Linear-by-Linear Association	.150	1	.698
N of Valid Cases	132		

a 5 cells (62.5%) have expected count less than 5. The minimum expected count is .09.

Appendix Table E29 Chi-square tests for relation between the metalaxyl sensitivities of isolates and the aggressive (lesion area) on infected potato leaflets cv. Spunta

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.674(a)	3	.879
Likelihood Ratio	1.029	3	.794
Linear-by-Linear Association	.072	1	.789
N of Valid Cases	132		

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is .36.

Appendix Table E30 Wilcoxon Signed Ranks Test for comparative test between technical and commercial grade of metalaxyl at 5 ppm

Ranks

		N	Mean Rank	Sum of Ranks
Commercial - Technical	Negative Ranks	6[a]	5.67	34.00
	Positive Ranks	4[b]	5.25	21.00
	Ties	0[c]		
	Total	10		

[a]C < T, [b]C > T, [c]C = T

Test Statistics[b]

	Commercial - Technical
Z	-.663[a]
Asymp. Sig. (2-tailed)	.508

[a]Based on positive ranks.

[b]Wilcoxon Signed Ranks Test.

Appendix Table E31 Wilcoxon Signed Ranks Test for comparative test between technical and commercial grade of metalaxyl at 100 ppm

Ranks

		N	Mean Rank	Sum of Ranks
Commercial - Technical	Negative Ranks	6[a]	4.83	29.00
	Positive Ranks	4[b]	6.50	26.00
	Ties	0[c]		
	Total	10		

[a]C < T, [b]C > T, [c]C = T

Test Statistics[b]

	Commercial - Technical
Z	-.153(a)
Asymp. Sig. (2-tailed)	.878

[a]Based on positive ranks.

[b]Wilcoxon Signed Ranks Test.

Appendix Table E32 Nucleotide bases of Intron Ras, Ras and B-Tubulin of *Phytophthora infestans* Thai isolates

Region	Isolate	Position		
		50	100	150
B-Tubulin	CMSS1-02	TACGACATTTGCTTCCGCACATTGAAGCTCACCACCCCCACTTATGGTGA	CCTGAACCACTTGGTGTGTGCCGCCATGTCCGGTATTACCACGTGCCTTC	GTTTCCCCGGTCAGCTGAACTCGGACCTGCGTAAGCTGGCCGTGAACCTG
		ATCCCGTTCCCGCGTCTCCACTTCTTTATGATTGGTTTCGCTCCTCTGAC	ATCGCGCGGCTCGCAGCAGTACCGCGCCCTGACGGTGCCCGAGCTGACCC	AGCAGCAGTTCGATGCTAAGAACATGATGTGTGCCGCCGACCCTCGCCAC
		GGCCGCTATTTAACTGCAGCTTGTATGTTCCGCGGACGCATGAGCACGAA	GGAGGTTGATGAGCAGATGCTGAACGTGCAGAACAAGAACTCGTCATACT	TCGTCGAGTGGATCCCCAACAACATCAAGGCTAGCGTGTGTGACATCCCG
		CCCAAGGGTCTGAAGATGAGCACTACGTTCATTGGTAACTCTACTGCTAT	CCAGGAGATGTTCAAGCG	
	CMSS2-08	TACGACATTTGCTTCCGCACATTGAAGCTCACCACCCCCACTTATGGTGA	CCTGAACCACTTGGTGTGTGCCGCCATGTCCGGTATTACCACGTGCCTTC	GTTTCCCCGGTCAGCTGAACTCGGACCTGCGTAAGCTGGCCGTGAACCTG
		ATCCCGTTCCCGCGTCTCCACTTCTTTATGATTGGTTTCGCTCCTCTGAC	ATCGCGCGGCTCGCAGCAGTACCGCGCCCTGACGGTGCCCGAGCTGACCC	AGCAGCAGTTCGATGCTAAGAACATGATGTGTGCCGCCGACCCTCGCCAC
		GGCCGCTATTTAACTGCAGCTTGTATGTTCCGCGGACGCATGAGCACGAA	GGAGGTTGATGAGCAGATGCTGAACGTGCAGAACAAGAACTCGTCATACT	TCGTCGAGTGGATCCCCAACAACATCAAGGCTAGCGTGTGTGACATCCCG
		CCCAAGGGTCTGAAGATGAGCACTACGTTCATTGGTAACTCTACTGCTAT	CCAGGAGATGTTCAAGCG	
	TKPP1-05	TACGACATTTGCTTCCGCACATTGAAGCTCACCACCCCCACTTATGGTGA	CCTGAACCACTTGGTGTGTGCCGCCATGTCCGGTATTACCACGTGCCTTC	GTTTCCCCGGTCAGCTGAACTCGGACCTGCGTAAGCTGGCCGTGAACCTG
		ATCCCGTTCCCGCGTCTCCACTTCTTTATGATTGGTTTCGCTCCTCTGAC	ATCGCGCGGCTCGCAGCAGTACCGCGCCCTGACGGTGCCCGAGCTGACCC	AGCAGCAGTTCGATGCTAAGAACATGATGTGTGCCGCCGACCCTCGCCAC
		GGCCGCTATTTAACTGCAGCTTGTATGTTCCGCGGACGCATGAGCACGAA	GGAGGTTGATGAGCAGATGCTGAACGTGCAGAACAAGAACTCGTCATACT	TCGTCGAGTGGATCCCCAACAACATCAAGGCTAGCGTGTGTGACATCCCG
		CCCAAGGGTCTGAAGATGAGCACTACGTTCATTGGTAACTCTACTGCTAT	CCAGGAGATGTTCAAGCG	
Ras	CMSS1-02	ACACGTACACGGAGAGCTACATCTCGACCATCGGTGTTGACTTTGTGAGT	GTCTAACATATTTTACGCCAAACGACCTTTTGTAAGGTCTAGATTGCCAT	TACATTGCTCACATGGCTTTCGCGATTTTGCTTAGAAAATCCGTACGATC
		GAGCTGGACGGCAAGACCATCAAGCTCCAAATTGTACGCCGCTAAAAAAA	CATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAA	CAGTGGGACACTGCCGGCCAGGAGCGTTTCCGCACGATCACTAGCAGTTA
		CTACCGCGGTGCCCACGGTATTATCGTGGTGTACGATGTGACGGACCAGG	AGTCGTTCAATAACGTGAAACAGTGGCTGCACGAGATCGATAGGTGCGCT	CGTCTTGTTTACGGAATGGACCTACAGAAAAGAAAGAGCTAAGTGATGGA
		CCGCTTTGATTTGCAGATACGCCTGTGAGAACGTGAACAAGCTGCTGGTT	GGTAACAAGAGCGATCTAACGGCCAAGCGCGTGGTGAGCA	
	CMSS2-08	ACACGTACACGGAGAGCTACATCTCGACCATCGGTGTTGACTTTGTGAGT	GTCTAACATATTTTACGCCAAACGACCTTTTGTAAGGTCTAGATTGCCAT	TACATTGCTCACATGGCTTTCGCGATTTTGCTTAGAAAATCCGTACGATC
		GAGCTGGACGGCAAGACCATCAAGCTCCAAATTGTACGCCGCTAAAAAAA	CATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAA	CAGTGGGACACTGCCGGCCAGGAGCGTTTCCGCACGATCACTAGCAGTTA
		CTACCGCGGTGCCCACGGTATTATCGTGGTGTACGATGTGACGGACCAGG	AGTCGTTCAATAACGTGAAACAGTGGCTGCACGAGATCGATAGGTGCGCT	CGTCTTGTTTACGGAATGGACCTACAGAAAAGAAAGAGCTAAGTGATGGA
		CCGCTTTGATTTGCAGATACGCCTGTGAGAACGTGAACAAGCTGCTGGTT	GGTAACAAGAGCGATCTAACGGCCAAGCGCGTGGTGAGCA	
	TKPP1-05	ACACGTACACGGAGAGCTACATCTCGACCATCGGTGTTGACTTTGTGAGT	GTCTAACATATTTTACGCCAAACGACCTTTTGTAAGGTCTAGATTGCCAT	TACATTGCTCACATGGCTTTCGCGATTTTGCTTAGAAAATCCGTACGATC
		GAGCTGGACGGCAAGACCATCAAGCTCCAAATTGTACGCCGCTAAAAAAA	CATTTGTCCCCGCGTGATTTCCTATTTAACTAACGGTTCTCCTATTTCAA	CAGTGGGACACTGCCGGCCAGGAGCGTTTCCGCACGATCACTAGCAGTTA
		CTACCGCGGTGCCCACGGTATTATCGTGGTGTACGATGTGACGGACCAGG	AGTCGTTCAATAACGTGAAACAGTGGCTGCACGAGATCGATAGGTGCGCT	CGTCTTGTTTACGGAATGGACCTACAGAAAAGAAAGAGCTAAGTGATGGA
		CCGCTTTGATTTGCAGATACGCCTGTGAGAACGTGAACAAGCTGCTGGTT	GGTAACAAGAGCGATCTAACGGCCAAGCGCGTGGTGAGCA	
IntronRas	CMSS1-02	TTGCAGCACAACCCAAGACGCTCAGAACGCGCGAGTCGCCTCGTTTTTCT	TCTCGGTGTAGGTGCCTTTCTATTCTTGTAGCGCCAATATGGGGCGCAGC	TGTCTCTCCCTTCATCTCCCCTCTTCTCGCCGGTCGCCCTCCCTTATCTA
		CACTGTATGGTAGAAGTGAATGGATCCTTGGTTTTTCTTTGGAATCTAGG	AGCGACGGAACGGGGAGAAGAAGGGAGCGTCATGGTATGCTGGAAGCTGT	CAACTAACGGGATCGCTGTGTCGTGCTGCAGATTGAGCGCCTTTACTCGC
		CGCTTTTTCTCTACATTCCACCAAGATGAACCCCGAATAGTACGTGCA		
	CMSS2-08	TTGCAGCACAACCCAAGACGCTCAGAACGCGCGAGTCGCCTCGTTTTTCT	TCTCGGTGTAGGTGCCTTTCTATTCTTGTAGCGCCAATATGGGGCGCAGC	TGTCTCTCCCTTCATCTCCCCTCTTCTCGCCGGTCGCCCTCCCTTATCTA
		CACTGTATGGTAGAAGTGAATGGATCCTTGGTTTTTCTTTGGAATCTAGG	AGCGACGGAACGGGGAGAAGAAGGGAGCGTCATGGTATGCTGGAAGCTGT	CAACTAACGGGATCGCTGTGTCGTGCTGCAGATTGAGCGCCTTTACTCGC
		CGCTTTTTCTCTACATTCCACCAAGATGAACCCCGAATAGTACGTGCA		
	TKPP1-05	TTGCAGCACAACCCAAGACGCTCAGAACGCGCGAGTCGCCTCGTTTTTCT	TCTCGGTGTAGGTGCCTTTCTATTCTTGTAGCGCCAATATGGGGCGCAGC	TGTCTCTCCCTTCATCTCCCCTCTTCTCGCCGGTCGCCCTCCCTTATCTA
		CACTGTATGGTAGAAGTGAATGGATCCTTGGTTTTTCTTTGGAATCTAGG	AGCGACGGAACGGGGAGAAGAAGGGAGCGTCATGGTATGCTGGAAGCTGT	CAACTAACGGGATCGCTGTGTCGTGCTGCAGATTGAGCGCCTTTACTCGC
		CGCTTTTTCTCTACATTCCACCAAGATGAACCCCGAATAGTACGTGCA		

Appendix Table E33 Prices of cereal grains used in this study obtained on January 2011

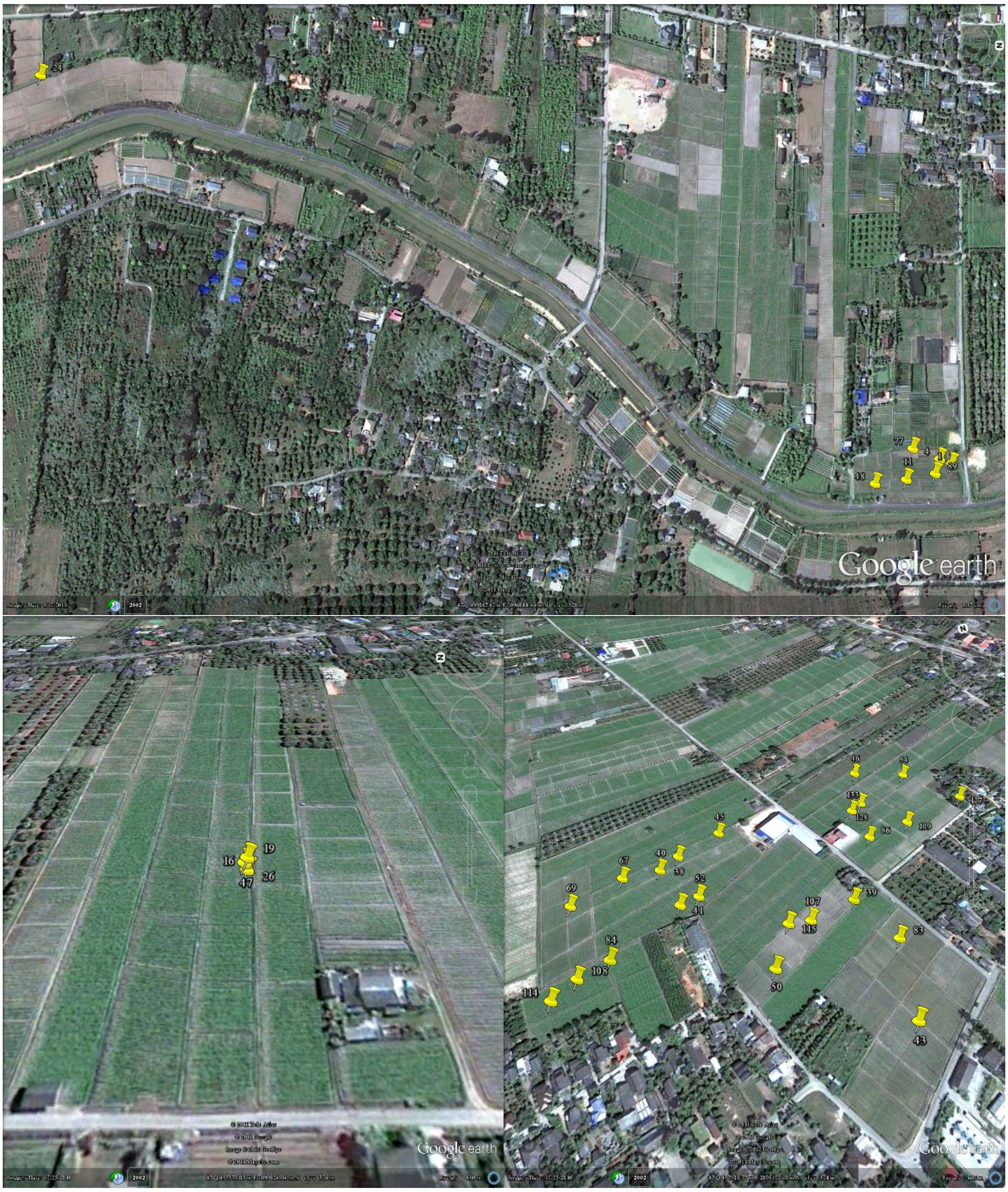
Common name	Scientific name	Price (Baht/kilogram)	Market place
Cereal grain			
Mung bean	*Phaseolus aureus* Roxb.	70.00	Department store
		30.00	Local market[a]
White sesame	*Sesamum orientale* L.	215.38	Department store
Black sesame	*Sesamum orientale* L.	254.54	Department store
Red kidney bean	*Phaseolus vulgaris* L.	50.00	Department store
Job's tear	*Coix lachrymal-jobi* L.	120.00	Department store
		70.00	Local market[b]
Barley	*Hordeum vulgare* L.	60.00	Local market[b]
Wheat	*Triticum aestivum* L.	100.00	Local market[b]
Peanut	*Arachis hypogaea* Linn.	67.00	Department store
Soybean	*Glycine max* Merr.	40.00	Department store
Dried maize	*Zea mays* L.	10.00	Local market[a]
Sunflower	*Helianthus annuus* L.	55.00	Local market[a]
Unhusked rice grain	*Oryza sativa* L.	11.00	Local market[a]
Black bean	*Phaseolus vulgaris* L.	48.00	Department store
White bean	*Phaseolus vulgaris* L.	278.00	Department store
Azuki bean	*Vigna angularis* (Willd.) Ohwi & H. Ohashi	60.00	Local market[a]
Common millet	*Panicum miliaceum*	133.00	Local market[b]
Sorghum	*Sorghum bicolor*	20.00	Local market[a]
Rye	*Secale cereale* subsp. *cereale*	25.00	www.amezon.com
Fresh product			
Fresh sweet corn	*Zea mays rugosa*	13.00	Local market
Waxy corn	*Zea mays ceratina*	15.00	Local market

[a]Commodity market

[b]Bio-organic food shop

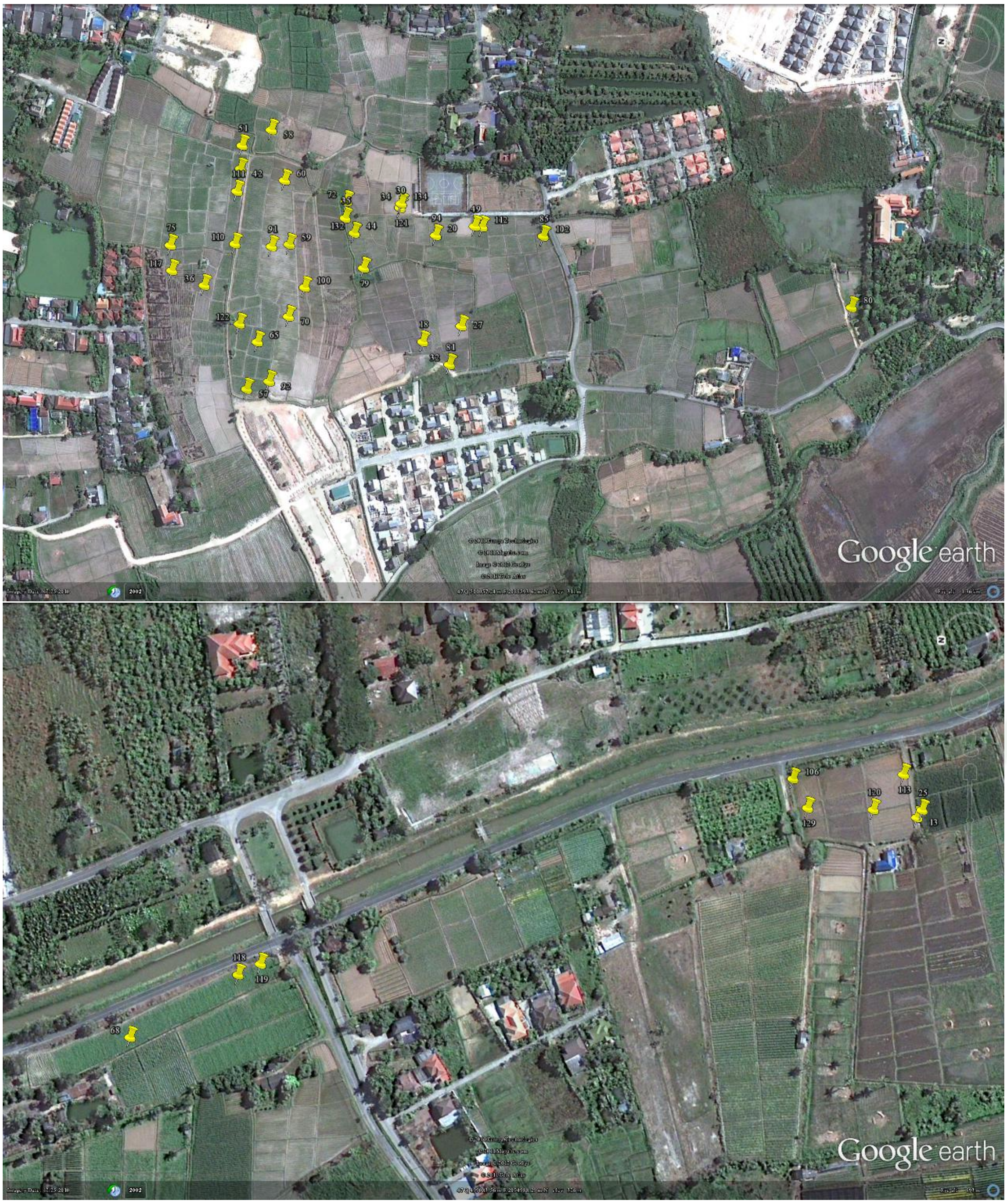
Appendix F

Figure



Appendix Figure F1 *Phytophthora infestans* CMSS1- isolates collected from Chedi MaeKrua Sub-District, SanSai District, ChiangMai Province

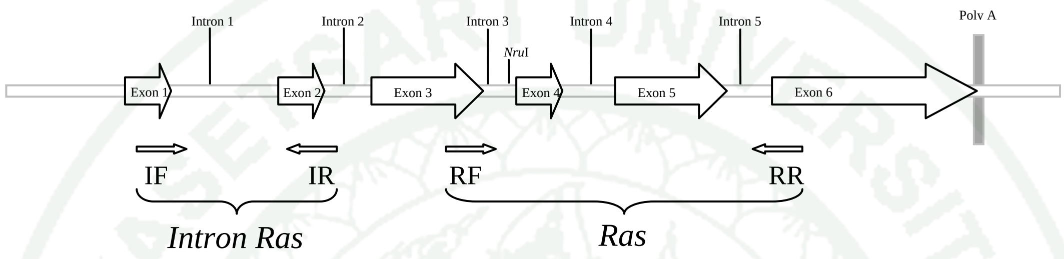
Appendix Figure F2 *Phytophthora infestans* CMSS2- isolates collected from Mae FaekMai Sub-District, SanSai District, ChiangMai Province



Appendix Figure F3 *Phytophthora infestans* CMSS3- isolates collected from Nong Han Sub-District, SanSai District, ChiangMai Province

Appendix Figure F4 *Phytophthora infestans* CMPr4- isolates collected from Long Khot Sub-District, Phrao District, ChiangMai Province

Appendix Figure F5 *Phytophthora infestans* TKPP1- isolates collected from Ruam Thai Phatthana Sub-District, Phob Phra District, Tak Province



Appendix Figure F6 *Phytophthora infestans Ras* gene organization showing location of primer and amplified regions, and the *Nru*I restriction site. (Gomez-Alpizar, 2004)

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