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Phenotypic and Genotypic Changes in the *Phytophthora infestans* Population in Taiwan – 1991 to 2006

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Abstract

Late blight, caused by *Phytophthora infestans*, is one of the most destructive diseases of tomato in Taiwan. A total of 655 isolates of *P. infestans*, including 29 isolates from potato, was collected from major tomato and potato production areas of Taiwan during 1991 to 2006. Isolates were characterized for their pathogenicity, mating type, *in vitro* metalaxyl sensitivity and molecular genotype (including allozyme pattern, mitochondrial genomic haplotype and DNA fingerprint) to monitor population changes in *P. infestans*. The population of *P. infestans* in Taiwan underwent a dramatic genetic shift in the 1997–1998 cool growing season. Isolates collected from tomato before 1997 were aggressive to tomato but not potato; most isolates obtained after 1998, were aggressive to both hosts. Metalaxyl sensitivity of isolates changed from sensitive/intermediate before 1997 to resistant since 1998. Similarly, the isolates obtained before 1997 were all US-1 clonal lineage (including variants US-1.1, US-1.2, US-1.3 and US-1.4). During the 1997–1998 cool growing season, the US-11 clonal lineage and the TW-1 genotype appeared, possibly introduced on imported table potatoes. The US-11 lineage spread rapidly and since 1999 has almost completely displaced the old population in Taiwan. Mating type determined by pairing with A1 and A2 reference isolages of *P. parasitica*, showed all isolates were of the A1 mating type, suggesting that the A2 mating type had not become established in Taiwan. The increasing percentage (up to 42.3% in 2006) of the US-11 variants (including US-11.l, US-11.2, US-11.3 and US-11.4) implied that genomic diversity of the pathogen is changing quickly. Therefore, it is important to continuously monitor the population changes of *P. infestans* and develop an integrated management strategy for this disease.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) De Bary, is a destructive disease of tomato and potato worldwide, especially during cool and moist weather conditions. *P. infestans* is a heterothallic Oomycete, which reproduces sexually when the A1 and A2 mating types interact. Prior to the 1980s, it was believed that a single asexual clone of the A1 mating type was distributed worldwide and that the A2 mating type and sexual reproduction were confined to highlands in Mexico (Fry et al., 1993). Disease outbreaks have increased in frequency and severity worldwide during the last two decades; this has been associated with the migration of new and more aggressive populations, including both mating types of the pathogen (Spielman et al., 1991; Fry et al., 1993; Fry and Goodwin, 1997). New populations of the pathogen carried resistance to metalaxyl which had been widely used to control this disease making management more difficult (Fry et al., 1992; Gisi and Cohen, 1996).

Late blight was reported in Taiwan on potato and tomato in the early 1900s (Kawakami and Suzuki, 1908; Sawada, 1919). Prior to 1997, late blight was a yearly threat in the Taiwan highlands, but it was not considered a major problem on potato or tomato production in lowland areas of the island. Since the 1997–1998 growing seasons, severe late blight epidemics have occurred on both tomato and potato crops in Taiwan (Ann et al., 1998; AVRDC, 1998), concomitant with the appearance of a new pathogen population (Ann and Chang, 2000; AVRDC, 2000; Jyan et al., 2004). A total of 139 *P. infestans* isolates collected by AVRDC-The World Vegetable Center in Taiwan from 1991 to 2001 showed that the *P. infestans* population changed dramatically (Deahl et al., 2002). Jyan et al. (2004) subsequently reported a similar conclusion based on 94 isolates collected in Taiwan from

1992 to 2002. Both Deahl et al. (2002) and Jyan et al. (2004) showed that a new genotype, US-11 clonal lineage, had been introduced into Taiwan which replaced the original US-1 genotype within a few years, but the population remained exclusively A1 mating type.

A2 mating type isolates have been detected in East Asian countries, including Japan in 1987 (Mosa et al., 1989) and Korea in 1991 (Choi et al., 1992), but not in Taiwan (Ho, 1990; Koh et al., 1994). The introduction of the A2 mating type would increase genetic recombination through sexual reproduction, and is therefore a concern for local quarantine systems (Sujkowski et al., 1994; Goodwin et al., 1995b; Mahuku et al., 2000; Knapova and Gisi, 2002). In this study, a long-term and large-scale survey of the *P. infestans* population on tomato and potato was conducted from 1991 to 2006 throughout the island of Taiwan. The aims of this study were: (i) to examine the mating type distribution of *P. infestans*, (ii) to monitor the phenotypic and genotypic changes in the *P. infestans* population and (iii) to relate these changes to the severity of recent late blight outbreaks in Taiwan.

Materials and Methods

Disease surveys and sample collection

Island-wide surveys of late blight to assess disease occurrence and severity, and to collect infected host tissues were conducted in 14 counties in Taiwan during the tomato and potato growing seasons from 1991 to 2006. Infected leaves, stems, or fruits of tomato (or potato) were collected from farmers' fields, home gardens, and research plots. One infected sample was selected from each sampling site for further study. The sample collection was included 139 isolates published previously by Deahl et al. (2002).

Isolation and preservation of the pathogen

After washing with tap water, infected host tissue was placed in a Petri dish on a piece of moistened filter paper at 20°C for 1–4 days to induce sufficient sporulation for isolation. Isolates were obtained by transferring fresh sporangia to Petri plates containing rye A agar amended with ampicillin (100 mg/l), nystatin (100 mg/l) and rifamipicin (50 mg/l) (Caten and Jinks, 1968), using a small agar block on the tip of a spatula (Deahl et al., 2002). The isolates were grown on rye A agar plates after isolation. Subsequently, isolates were maintained on rye A agar slants covered with mineral oil and also in test tubes containing autoclaved rye grains and distilled water at 16–20°C.

Host specificity

Inoculum was prepared by washing with rubbing sporangia from colonies grown on rye A agar plates with sterile distilled water. Sporangial suspensions were adjusted to a concentration of 5×10^4 sporangia/ml using a hemacytometer and then incubated at 12°C for 3 h to induce zoospore release. The zoospore/sporangia suspension of each isolate was atomized with a commercial paint sprayer to the point of run-off onto six tomato seedlings (AVRDC line CL5915, *Solanum lycopersicum*) at the four–five true leaf stage and six potato plants (Kennebec, *S. tuberosum*) at the five–six true leaf stage. There were three replications of six plants for both potato and tomato. Tomato line CL5915 and potato cultivar Kennebec do not contain any *Ph* or *R* resistance gene to *P. infestans*, respectively. Plants atomized with water were used as controls. Inoculated plants were incubated at 100% RH and 20 ± 2°C without light for the first 24 h. Thereafter, inoculated plants were held at 60–95% RH and 20 ± 2°C with a 14 h light (70 $\mu E/m^2/s$) period per day. Disease severity on tomato and potato was evaluated 7–10 days after inoculation with each isolate by visually scoring severity on a scale of 0–6 where 0 indicated no symptom; 1 indicated 1–5% leaf area affected, small lesions; 2 indicated 6–15% leaf area affected, restricted lesions; 3 indicated 16–30% leaf area affected and/or water-soaked flecks on stems; 4 indicated 31–60% leaf area affected and/or a few stem lesions; 5 indicated 61–90% leaf area affected and/or expanding stem lesions and 6 indicated 91–100% of leaf area affected and/or extensive stem damage, or plant dead (AVRDC, 1998).

Determination of mating type

Mating type of *P. infestans* isolates was determined by pairing each isolate with known A1 (isolate P991) and A2 (isolate P731) mating type reference cultures of *P. parasitica* on rye A agar plates. Paired cultures were incubated at 20 ± 1°C in the dark for 10–14 days and then examined microscopically for the presence of oospores/oogonia. If oospores/oogonia were seen on the plate with the known A1 isolates, but not on the plate with the known A2 isolate, then the test isolate was A2 mating type. If the opposite was seen, the test isolate was A1 mating type (Hohl and Iselin, 1984).

In vitro assessment of metalaxyl sensitivity

Sensitivity to metalaxyl was determined by the growth response of each isolate on rye A agar plates amended with metalaxyl (Matuszak et al., 1994). Metalaxyl (100 ppm) was added to rye A medium at 50°C just prior to dispensing it into Petri dishes. Agar blocks (4-mm diameter) with active mycelia were taken from the colony margin of each isolate and transferred to the centre of three replicate plates of metalaxyl-amended rye A agar. Growth of the same isolates on rye A agar plates without metalaxyl served as controls. Radial growth of each isolate was measured after incubation in darkness at 20 ± 1°C for 7 days and the mean of the three replicates calculated. Mean percentage growth of each isolate on metalaxyl-amended agar compared with growth on unamended agar was calculated. Metalaxyl-sensitive, metalaxyl-intermediate, and metalaxyl-resistant isolates were defined as those with < 10%, > 10% but < 60% and > 60% growth compared to the control, respectively (Shattock, 1988).

Molecular genotyping

Molecular genotype characterization of each isolate followed that of the *Phytophthora infestans* global marker database (Forbes et al., 1998).

Genotypes with two polymorphic allozyme loci, *Glucose-6-phosphate isomerase* (*Gpi*) and *Peptidase* (*Pep*), were revealed following electrophoresis and staining with the appropriate agar overlays on cellulose acetate plates (Goodwin et al., 1995a).

The mtDNA haplotypes of the isolates were determined by PCR-RFLP using a modification of Griffith and Shaw (1998). P2 and P4 polymorphic fragments of mitochondrial DNA were amplified by two oligonucleotide primers, F2/R2 and F4/R4 and then digested by *Msp*I and *Eco*RI, respectively. Digested DNA patterns were resolved by electrophoresis at approximately 10 V/cm for 1–1.5 h and visualized using a UV transilluminator at 254 nm.

DNA RFLP-fingerprinting was carried out using the moderately repetitive probe, RG57. The genomic DNA of *P. infestans* was digested by *Eco*RI, transferred to Hybond N+ Nylon membrane and then hybridized by the RG57 probe labelled with fluorescein. An autoradiography film was developed according to the standard techniques to visualize the polymorphic band hybridized with the labelled probe (Goodwin et al., 1992a).

All *P. infestans* isolates used in this study were compared with isolates collected from other countries at the USDA-ARS Vegetable Laboratory at Beltsville, MD, USA.

Results

Disease survey and sample collection

Since 1998, devastating outbreaks of tomato and potato late blight have occurred yearly in highland (500+ meters elevation) and eastern areas of Taiwan causing severe economic losses. Outbreaks have been associated with lower night temperatures and higher relative humidity during the growing seasons. In total, 655 isolates, including 29 from potato were collected during island-wide sampling (Fig. 1)

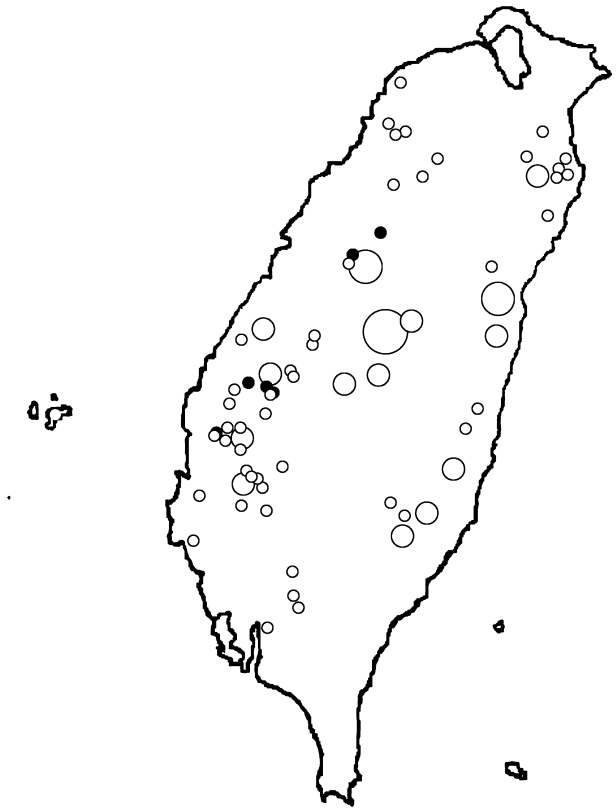

Fig. 1 Locations of *Phytophthora infestans* isolates collected in Taiwan from 1991 to 2006. Solid black circles indicate potato isolates while open circles indicate tomato isolates. The size of the circles corresponds to the number of isolates collected at that location

Table 1
Pathogenicity of *Phytophthora infestans* isolates collected in Taiwan from 1991 to 2006

Years	No. of isolates collected	Host	Virulence[a]	
			Tomato	Potato
1991–1997	28 (US-1)	Tomato	+	–
1998–2006	8 (US-1)	Tomato	+	–
	590 (US-11)	Tomato	+	+
	29 (US-11)	Potato	+	+

[a]Virulence of the fungal isolates was determined according to visual symptoms by cross-infection on both tomato (AVRDC line CL5915, *S. lycopersicum*) and potato (Kennebec, *S. tuberosum*).

Host specificity

All 28 isolates collected from tomato from 1991 to 1997 were aggressive to tomato but not potato. Host specificity of *P. infestans* isolates changed markedly after a severe outbreak in the 1997–1998 growing seasons (Table 1). All but eight of 627 isolates collected from either tomato or potato from 1998 to 2006 were highly virulent on both crops.

Determination of mating type

All *P. infestans* isolates were identified at AVRDC as A1 mating type after pairing with reference isolates of both mating types of *P. parasitica* for 2 weeks.

***In vitro* assessment of metalaxyl sensitivity**

Growth responses on metalaxyl-amended agar indicated that metalaxyl sensitivity of *P. infestans* isolates in Taiwan changed from sensitive/intermediate to resistant beginning in 1998 (Table 2). None of the isolates collected before 1998 was resistant to metalaxyl with 46% and 54% of these isolates expressing metalaxyl-sensitive and metalaxyl-intermediate reactions, respectively. After 1998, the vast majority of the isolates collected was metalaxyl resistant with only two and 11 isolates expressing metalaxyl sensitive and metalaxyl intermediate reactions, respectively (Table 2).

Molecular genotype characterization of *Phytophthora infestans*

Four dilocus allozyme genotypes were determined by cellulose acetate electrophoresis and overlay staining. Four allozyme patterns were characterized as follows:

Table 2
Metalaxyl sensitivity[a] of *Phytophthora infestans* isolates collected from Taiwan from 1991 to 2006

Collected years	Total no of isolates tested	No. (%) of isolates in the category		
		Resistant	Intermediate	Sensitive
1991—1997	28	0 (0.0)	15 (53.6)	13 (46.4)
1998	33	27 (81.8)	2 (6.1)	4 (12.1)
1999	24	22 (91.7)	1 (4.2)	1 (4.2)
2000	37	34 (91.9)	3 (8.1)	0 (0.0)
2001	58	58 (100.0)	0 (0.0)	0 (0.0)
2002	58	56 (96.6)	2 (3.4)	0 (0.0)
2003	101	99 (98.0)	1 (1.0)	1 (1.0)
2004	113	113 (100.0)	0 (0.0)	0 (0.0)
2005	106	102 (96.2)	4 (3.8)	0 (0.0)
2006	97	97 (100.0)	0 (0.0)	0 (0.0)
Total	655	608 (92.8)	28 (4.3)	19 (2.9)

[a]Metalaxyl sensitivity was determined by amended-agar assays (Matuszak et al., 1994) at the concentration of 100 parts per million. Metalaxyl-sensitive, intermediate and resistant isolates were defined as less than 10%, more than 10% but less than 60%, and more than 60% growth response, respectively (Shattock, 1988).

(i) 25 isolates were *86/100* and *92/100*; (ii) 11 isolates were *86/100* and *100/100*, (iii) 615 isolates were *100/100/111* and *100/100* and (iv) four isolates were *100/100/122* and *100/100*, for the loci coding for *Gpi* and *Pep*, respectively (Fig. 2 and Table 3).

Two mitochondrial DNA haplotypes were revealed through PCR-RFLP analyses of the polymorphic regions of the mitochondrial genome – 36 and 619 isolates had the Ib and IIb mtDNA haplotypes, respectively (Fig. 2 and Table 3). These characteristics together with the RG57 fingerprints indicated that the isolates separated into US-1 and US-11 clonal lineage groups (Fig. 2 and Table 3).

All isolates collected before 1998 had the dilocus allozyme genotypes *86/100* and *92/100* (or *100/100*) for *Gpi* and *Pep*, respectively, and were mitochondrial haplotype Ib. These characteristics together with their RG57 fingerprints, indicated that these isolates belonged to the US-1 clonal lineage including variants US-1.1, US-1.2, US-1.3 and US-1.4. However, the new genotype detected from 1998 on had the dilocus allozyme genotype *100/100/111* and *100/100* for *Gpi* and *Pep,* respectively, and the IIb mtDNA haplotype. Its attribution to the US-11 genotype was confirmed by the RG57 fingerprints.

Variants of US-11 included isolates identical with US-11, except for (i) lacking RG57 band 5 (US-11.1 previously reported by Deahl et al., 2002), (ii) lacking RG57 band 10 and having band 12 present (designated US-11.2), (iii) lacking RG57 band 10 (designated US-11.3) and (iv) lacking RG57 band 18 (designated US-11.4). Among the variants, US-11.1 and US-11.2 occurred most frequently (152/587) while US-11.3 and US-11.4 occured much less frequently (11/587) within the survey period. The US-11 variants were assumed to be members of a single clonal lineage derived from the US-11 genotype (Tables 3 and 4).

The four isolates containing *Gpi* alleles *100/100/122* were identified only in 1999 (one isolate) and 2004 (two isolates). They were identical with the US-11 genotype in terms of their *Pep* genotype, mtDNA haplotype and RG57 fingerprint and designated as TW-1 (Tables 3 and 4).

Most isolates (587/594) collected after 1998 were characterized as belonging to US-11 clonal lineage. The new lineage spread quickly and became predominant. The US-1 clonal lineage was dramatically replaced by US-11 during the 1998 growing season. Among all isolates collected from 1998 through 2006, only five collected in 1998 were of the US-1 clonal lineage and thereafter only single isolates in 1999, 2002 and 2003. Only one isolate collected in 1999 and three isolates in 2004 were of the TW-1 genotype (Table 4).

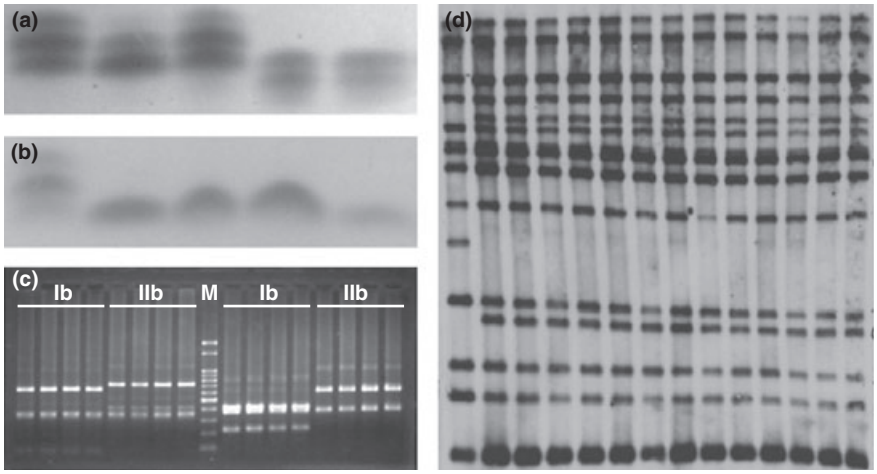

Fig. 2 Molecular genotype characterization of *Phytophthora infestans* isolates collected in Taiwan from 1991 to 2006. (a) The isozyme polymorphic pattern for *Glucose-6-phosphate isomerase* loci (*Gpi*). Lanes 1–3 showed the pattern of alleles *86/100*, and lanes 4 and 5 showed alleles *100/100/111* (Goodwin et al., 1995a). (b) The isozyme polymorphic pattern for *Peptidase* loci (*Pep*). Lane one was allele *92/100*, and the others were *100/100* (Goodwin et al., 1995a). (c) The PCR-RFLP pattern for mitochondrial haplotyping. Polymorphic regions were amplified from mitochondrial DNA of *P. infestans*. Lanes 1–8 were amplified by primer pair F2/R2 then digested by *Msp*I, lane 9 was 100 bp marker and lanes 10–17 were amplified by F4/R4 then digested by *EcoR*I (Griffith and Shaw, 1998). (d) RFLP-fingerprinting pattern hybrid by RG57 probe. Lane one was identified as US-1 genotype and the others were US-11 (Goodwin et al., 1992a)

Table 3
Summary of genotypes of *Phytophthora infestans* identified in Taiwan from 1991 to 2006

Genotype[a]	Mating type	Metalaxyl sensitivity[b]	Allozyme genotype		mtDNA haplotype[e]	RG57 fingerprint[f]	Comments
			Gpi[c]	*Pep*[d]			
US-1	A1	S/I	*86/100*	*92/100*	Ib	10101010110011010000110011	Old established genotype possibly present before the 1900s.
US-1.1	A1	S/I	*86/100*	*100/100*	Ib	1010101011001101000110011	A variant that is different from the US-1 by having 100/100 alleles at the *Pep* loci. Seven isolates from Nantou, two from Tainan, and one from Ilan counties during 1994–1998.
US-1.2	A1	S/I	*86/100*	*92/100*	Ib	10101010100011010000110011	A variant that is different from the US-1 by lacking RG57 fingerprint band 10. Two isolates from Nantou county in 1991 and 1996, respectively.
US-1.3	A1	S	*86/100*	*92/100*	Ib	10101010010011010000110011	A variant that is different from the US-1 by lacking RG57 fingerprint band 9. Two isolates from Tainan county in 1994 and 1997 respectively.
US-1.4	A1	S	*86/100*	*100/100*	Ib	10101010100011010000110011	A variant that is different from the US-1 by having 100/100 alleles at the *Pep* loci and lacking RG57 fingerprint band 10. One isolate from Chayi county in 1997.
US-11	A1	I/R	*100/100/111*	*100/100*	IIb	10101110010011010110011	Immigrant from imported potatoes during 1997–1998. It is the predominant population detected in Taiwan recently.
US-11.1	A1	I/R	*100/100/111*	*100/100*	IIb	10100110010011010110011	A variant that is different from the US-11 by lacking RG57 fingerprint band 5. Forty-four isolates from six counties during 1998–2006.
US-11.2	A1	R	*100/100/111*	*100/100*	IIb	10101110000111010110011	A variant that is different from the US-11 by lacking RG57 fingerprint band 10 and having band 12 present. One hundred and eight isolates from eleven counties during 1998–2006. It is the predominant genotype of the US-11 variants and increasing in recent years.
US-11.3	A1	R	*100/100/111*	*100/100*	IIb	10101110000011010110011	A variant that is different from the US-11 by lacking RG57 fingerprint band 10. Nine isolates from five counties, three in 1998 and six in 2005, respectively.
US-11.4	A1	R	*100/100/111*	*100/100*	IIb	10101110010011010000110011	A variant that is different from the US-11 by lacking RG57 fingerprint band 18. One isolate from Zhanghua in 1998 and one from Pingdong in 2005, respectively.
TW-1	A1	R	*100/122*	*100/100*	IIb	10101110010011010110011	Probable a sister clone of US-11 genotype immigrated from imported potatoes during 1997 to 1998. One isolate from Tainan county in 1998. Two isolates from Yunlin and one from Zhanghua counties in 2004, respectively.

[a]Variants of a lineage are indicated by appending a period and a sequential number after the genotype name. Therefore, US-1.1 genotype is the first variant identified within the US-1 clonal lineage. These variants are identical to the reference clone except for one or two variations at allozyme or DNA fingerprint loci. They are assumed to have arisen from asexual variation within lineages (Forbes et al., 1998); [b]Metalaxyl sensitivity was determined by amended-agar assays at a concentration of 100 parts per million. S, sensitive; I, intermediate and R, resistant were according to the growth response on the metalaxyl-amended media compared to the metalaxyl-free media (Shattock, 1988); [c]*Glucose-6-phosphate isomerase*; [d]*Peptidase;* [e]Mitochondrial haplotypes were revealed through PCR amplification and RFLP analysis of polymorphic regions of the mitochondrial genome (Griffith and Shaw, 1998); [f]Presence (1) or absence (0) of RG57 fingerprint bands 1–25 from left to right (band 4 is not reproducible and should not be used as genotype characterization) (Goodwin et al., 1992a).

Discussion

Destructive late blight epidemics in Taiwan were observed after 1998 and associated with an introduced pathogen lineage-US-11. The US-11 genotype was first reported in the Columbia Basin of Oregon and Washington in 1993 (Miller et al., 1997) and detected as predominated in populations in western Washington during 1996–1997 (Dorrance et al., 1999) and in California during 1998 (Gavino et al., 2000), respectively. Since 1995, Taiwan has imported potatoes

Table 4
The population shift of *Phytophthora infestans* isolates collected in Taiwan from 1991 to 2006 according to the multi-locus genotype characterization

	Number of isolates with multi-locus genotypes based on global markers[a]							
Collected years	US-1	US-1.1	US-1.2	US-1.3	US-1.4	US-11	US-11.v[b]	TW-1[c]
1991–1997	15	8	2	2	1	0	0	0
1998	3	2	0	0	0	20	7	1
1999	1	0	0	0	0	19	4	0
2000	0	0	0	0	0	33	4	0
2001	0	0	0	0	0	52	6	0
2002	1	0	0	0	0	41	16	0
2003	1	0	0	0	0	83	17	0
2004	0	0	0	0	0	79	31	3
2005	0	0	0	0	0	69	37	0
2006	0	0	0	0	0	56	41	0

[a]Multilocus genotypes of the *Phytophthora infestans* isolates were examined including mating type, metalaxyl sensitivity, allozyme genotype, mitochondrial DNA haplotype and RFLP-fingerprinting by RG57 probe (Forbes et al., 1998); [b]US-11v genotype isolates were detected for identical multi-locus polymorphisms pattern with US-11 genotype, except the variant on band 5, 10, 12 and 18; [c]TW-1 genotype isolates were detected for identical multi-locus polymorphisms pattern with US-11 genotype, except the variant on the *100/100/122* alleles at *Gpi* loci. They were probable a recombinant from the same parent with the US-11 lineage.

only from the United States with most coming from Washington State. It has been suggested that the US-11 lineage may have been introduced on latently infected table potatoes from North America (Deahl et al., 2002; Jyan et al., 2004). The introduced US-11 genotype became established during the 1997–1998 growing seasons and has almost completely displaced the US-1 *P. infestans* population in recent years (Table 4). Concomitant with this was a shift in metalaxyl sensitivity of the pathogen population from sensitive/intermediate to resistant (Table 2). Metalaxyl was an effective fungicide for the control of late blight before 1998 in Taiwan. Results of this study imply that farmers can no longer rely on metalaxyl for late blight control in Taiwan.

A2 mating type isolates have been found in many parts of the world outside Mexico since the second worldwide migration of *P. infestans* during the 1980s (Spielman et al., 1991). None of the isolates in this study nor other previous studies in Taiwan (Ann & Chang, 2002; Deahl et al., 2002; Jyan et al., 2004) have been found to be of the A2 mating type, suggesting the A2 mating type isolates may not have been introduced into Taiwan up to 2006. This conclusion is supported by previous studies in the United States that reported occurrence of only the A1 mating type among isolates of US-11 clonal lineage (Dorrance et al., 1999; Goodwin et al., 1998; Miller et al., 1997). However, upon retesting of Taiwan isolates from the current study in the USDA-ARS lab, one from the 2004 collection and another one from the 2006 collection were found to be of the A2 mating type (Deahl et al., 2008). The reason for conflicting results in the two labs is unclear, but may be related to the use of different mating type reference cultures, i.e. *P. parasitica* at AVRDC and *P. infestans* at USDA-ARS. Nonetheless, identification of a low frequency of A2 isolates from Taiwan now becomes a major concern as to what impact they may be having on current populations of *P. infestans* in Taiwan or may have in the future. All US-11 lineage isolates collected since 1998 were highly aggressive to both tomato and potato. This is a major difference between the *P. infestans* population in Taiwan and those of most other regions worldwide where, in general, more aggressive *P. infestans* isolates have been accompanied by introduction of the A2 mating type (Spielman et al., 1991; Koh et al., 1994; Goodwin et al., 1998; Cohen, 2002). The results also indicate that the host specificity of the pathogen populations in Taiwan changed from 1998 (Table 1). Twenty-eight US-1 isolates collected from tomato before the severe outbreak of 1998 were aggressive to tomato but not to potato; whereas, all isolates other than those of US-1 lineage collected from tomato after the severe outbreaks were aggressive to both hosts.

Reports on host specificity of *P. infestans* populations isolated from different hosts are inconsistent. The *P. infestans* populations from tomato and potato were characterized as the same genotypes in some regions of the Netherlands (Fry et al., 1991) and North America (Goodwin et al., 1995b; Legard et al., 1995). In contrast, distinct genotypes were reported associated with different hosts in northwestern Mexico (Goodwin et al., 1992b), the Philippines (Koh et al., 1994), Ecuador (Oyarzun et al., 1998) and one region of the Netherlands (Fry et al., 1991). Oyarzun et al. (1998) reported that isolates collected from Ecuador could be separated into a tomato population and a potato population with distinct genotypes, US-1 and EC-1, respectively. The EC-1 genotype was introduced into Ecuador and replaced the US-1 on potato but not tomato. This differs from results of the present study in which the US-11 genotype replaced the US-1 genotype in both potato and tomato. Oyarzun pointed out that no *P. infestans* isolates were found to be highly aggressive on both hosts; whereas, the US-11 lineage *P. infestans* isolates in our study are aggressive to both tomato and potato in the same geographical area. This is in agreement with the study conducted in western Washington in 1996 in which US-11 isolates were collected from tomato, potato, hairy nightshade and bittersweet (Dorrance et al., 1999).

The dramatic population shift of *P. infestans* isolates in Taiwan might have resulted from the widespread distribution of potatoes contaminated with the US-11 lineage, or more possibly, from the higher environmental fitness of the new lineage which led to it spreading rapidly from an established locus. Ann and Chang (2000) reported that the US-11 lineage isolates collected in Taiwan grew faster in culture and could withstand higher temperatures than the old US-1 lineage. Additionally, Dorrance et al. (1999) suggested the wilder host range of the US-11 genotype may give it a fitness advantage, which may allow it to dominate the populations. The apparent failure of the TW-1 genotype to become established in Taiwan might result from its lower aggressiveness to tomato (AVRDC, unpublished data). Metalaxyl resistance of the US-11 genotype might also have served as a driver for its spread. In addition, weather conditions during the 1997–1998 growing seasons (three times more rainfall compared with the average) also contributed to the severity of the epidemics and may have assisted in dissemination and establishment of the new strain (Deahl et al., 2002). Introduction of the US-11 clonal lineage and TW-1 genotype into Taiwan and the occurrence of the TW-1 genotype seem to have been the result of a unique event because they appear to have no relationship with *P. infestans* populations recently introduced into other Asian countries (Koh et al., 1994).

The US-11 clonal lineage has been predominant in the Taiwan population of *P. infestans* in recent years; however, one US-1 lineage isolate was detected in each of the years 1999, 2002 and 2003. These US-1 isolates were all obtained from tomato grown on the Puli Branch Station of the Taichung District Agricultural Improvement Station located in Central Taiwan at 500 m elevation. Although the US-1 lineage of *P. infestans* has not been detected since 2004, it cannot be concluded that it is extinct in Taiwan. Modified sampling strategies might improve the understanding of the pathogen population structure in Taiwan, for example increasing numbers of samples from a single field and sampling from isolated fields separated from other Solanaceous crops.

This study is the first that surveyed late blight throughout most of the tomato and potato production areas of Taiwan and examined large numbers of samples over a period of 16 years. Studies of the pathogen population structure over time will provide information on its evolutionary history which can help in the effective deployment of host resistance and fungicides for disease management.

Although sexual recombination has been observed and played a role in increasing genetic diversity in *P. infestans* populations in several countries (Sujkowski et al., 1994; Goodwin et al., 1995b, 1998; Mahuku et al., 2000; Knapova and Gisi, 2002), data from this study implied migration and asexual reproduction were the predominant mechanisms for the population shift of *P. infestans* in Taiwan.

Besides the variants of the US-1 clonal lineage, a significant number of *P. infestans* isolates were characterized as US-11 variants with one or two variations at DNA fingerprint loci. The US-11 variant lacking fingerprint band 5 has previously been designated as US-11.1. The US-11 variants (i) lacking RG57 fingerprint band 10, (ii) lacking band 10 with band 12 present and (iii) lacking band 18 were designated as US-11.2, US-11.3 and US-11.4, respectively. Apart from the introduction of the US-11 clonal lineage and the occurrence of the TW-1 genotype, no new clonal lineage of *P. infestans* isolates was detected in Taiwan during 1991 to 2006; however, the increasing incidence of variants of the US-11 clonal lineage implies that the genetic basis of the pathogen population in Taiwan is changing quickly (Table 4). Further studies of *P. infestans* isolates using additional molecular markers (Cooke and Lees, 2004) may allow a better understanding of genetic variability of the *P. infestans* population in Taiwan.

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