SSR assessment of Phytophthora infestans populations on tomato and potato in British gardens demonstrates high diversity but no evidence for host specialisation

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Abstract

Phytophthora infestans populations can differ in composition as a result of host-specialisation on tomato and potato hosts. In Great Britain many amateur gardeners grow outdoor tomatoes but there is little or no commercial tomato production outdoors. This study analysed isolates of *P. infestans* from British gardens with twelve multiplexed SSR markers that are used to monitor the disease on commercial potato crops. Samples of *P. infestans* from tomato hosts were collected in three years and from potato in one year from across Great Britain. Seven previously unreported genotypes were detected in garden populations and higher frequencies of unique clonal lineages (28% to 40%) were present compared with populations from British commercial potato crops reported elsewhere. Garden populations had a lower proportion (11% to 48% less) of the most common lineages (13_A2 and 6_A1) which together made up at least 86% of the commercial potato populations during the sampling

period. Host species accounted for only 2.6% of molecular variance detected between garden potato- and tomato-hosted samples. No significant difference in clonal lineage composition was found between host species in Great Britain and this could be due to the whole *P. infestans* population over-wintering on potato. British garden populations on both hosts were much more diverse than those on commercial potato crops; this finding may be influenced by less frequent fungicide use by gardeners and a higher diversity of un-sprayed susceptible potato cultivars enabling metalaxyl-sensitive and less virulent genotypes to survive in gardens.

Introduction

Late-blight caused by *Phytophthora infestans* is one of the most serious diseases of crops of potato (*Solanum tuberosum*) and outdoor tomato (*S. lycopersicum*) worldwide, causing yield reductions and increased production costs associated with fungicide sprays in potato. Late blight can cause 100% crop loss in tomato, particularly in unsprayed organic crops. *P. infestans* is capable of sexual and asexual reproduction. For sexual reproduction (and genetic recombination) A1 and A2 mating type genotypes must come into contact. In many areas with or without both mating types present, including Great Britain, reproduction is mostly or entirely asexual (Cooke *et al.* 2014; Collins, 2013; Lees *et al.* 2012). In these situations, recurrent clonal lineages dominate in *P. infestans* populations, with little or no genetic change from parent to offspring generations. Accumulation of mutations within clonal lineages leads to intra-lineage variation (Cooke *et al.* 2014). Novel clonal lineages may arise in agricultural systems through more extensive mutation, mitotic recombination, sexual recombination, or migration from *P. infestans* diversity hotspots such as South America (Goodwin *et al.* 1994).

P. infestans is thought to have originated in Mexico and spread to the South American Andes early in its evolution (Goss *et al*. 2014). It remained within this range until the North American and European potato blight epidemics of the 1840s (Bourke 1964), which seem to have been caused by a single *P. infestans* clonal lineage, HERB-1 (Yoshida *et al*. 2013). HERB-1 was displaced by the closely related US-1 lineage (Yoshida *et al*. 2013), which was subsequently the only clonal lineage detected outside South America and Mexico (Goodwin *et al.* 1994) until the next migration event in the late 1970s, which introduced new genotypes (including those with A2 mating types) from Mexico to Europe and subsequently to the rest of the world (Hohl & Iselin 1984; Spielman *et al.* 1991). With the migration of A2 mating types in the late 1970s came the potential for sexual recombination and the rapid genetic

diversification of the global *P. infestans* population. Thus, since the early 1980s, *P. infestans* populations in most European countries have consisted of multiple unique genotypes and recurrent clonal lineages (Euroblight 2014).

P. infestans is capable of infecting several species of Solanaceae worldwide, with *Solanum* species being the most frequent hosts (Nelson 2008). Of the species susceptible to *P. infestans,* potato and tomato are the two most economically important and widespread. In many global regions where multiple *P. infestans* genotypes and multiple host species are present, some degree of host-specialisation has been observed, both in terms of the frequency of recurrent clonal lineages on each host, and in terms of within-lineage genotypic and phenotypic variation. For example, multiple studies in the USA have demonstrated that the US-8 lineage is common on potato, but very seldom infects tomato (Danies *et al.* 2012; Peters *et al.* 2014; Wangsomboondee *et al.* 2002). In studies where inoculations onto tomato and potato leaflets have been carried out, isolates are often found to be more pathogenic on one host than another. This specialisation may correspond to the *P. infestans* clonal lineage (Danies *et al.* 2012) or to the host species from which the isolate was originally collected (Garry *et al.* 2005; Knapova and Gisi 2002; Lebreton *et al.* 1999; Oyarzun *et al.* 1998).

P. infestans is a serious threat to British potato production, and *P. infestans* populations in Great Britain have been closely monitored on commercial potato crops since the mid-1990s (Cooke *et al.* 2014; Cooke *et al.* 2007; Day *et al.* 2004). This work has shown that since 2007-2008, two strains, 13_A2 and 6_A1, have become dominant in most areas of Great Britain, with relatively few other clonal lineages present (Cooke *et al.* 2014). In Great Britain, there is little or no commercial outdoor tomato cultivation, and the glasshouse tomato crop is rarely blighted due to the warmer, dryer conditions in glasshouses (Collins 2013; Nelson 2008). However, amateur gardeners commonly grow outdoor tomato crops and experience late-blight outbreaks on these as well as on potato. In Great Britain most monitoring of *P. infestans* populations has taken place in commercial potato crops, and little is known about the *P. infestans* population on tomato, or the diversity of *P. infestans* present in domestic gardens and allotments (henceforth "gardens"). However, with over 150,000 allotment plots in England (Campbell & Campbell 2013), and outdoor tomatoes widely grown by amateur gardeners throughout Great Britain, there is potentially additional *P. infestans* diversity that has not yet been sampled. It is hypothesised that there might be distinct tomato and potato-specialised populations in line with the situation in other regions. Since it has been suggested that gardens could be an important source of inoculum for commercial potato crops (Ball & Stevenson 2012), understanding more about the structure of *P. infestans* populations in these settings is vital.

This study was conducted to compare populations of *P. infestans* from tomato and potato for genetic variation at SSR (microsatellite) loci and to examine the diversity of *P. infestans* genotypes present in gardens, as distinct from commercial potato crops, in Great Britain between 2011 and 2013.

Materials and Methods

Isolation of P. infestans *from Gardens*

P. infestans samples were obtained from private gardens and allotment sites throughout Great Britain by appealing through several gardening websites and magazines and by directly contacting allotment organisations or public administrators in 300 major British cities and regions. In 2011 and 2012, *P. infestans* samples were sought from tomato only. In 2013, samples were sought from both tomato and potato crops. Responding groups and individuals were asked to mail fresh, otherwise healthy leaflets bearing small lesions to the authors.

Upon receipt, samples were placed in a 9 cm diameter Petri dish lined with damp tissue paper and incubated at cool room temperature (15-20 $^{\circ}$C) in diffuse natural light for 24 hours. Once sporulation was observed, an agar wedge was used to transfer sporangia to a plate containing Rye A medium (Caten & Jinks 1968) amended with 25 mg L^{-1} of rifampicin and ampicillin (both Bio Basic Canada Inc.) and 50 mg L^{-1} of nystatin (Sigma Aldrich). The cultures were incubated in darkness at 18 $^{\circ}$C in order to grow mycelium from which DNA was extracted. For some isolates, sporangia would not germinate on agar, and in this case DNA was extracted directly from infected plant material.

In 2011 and 2012 respectively, 15 and 36 usable tomato-hosted isolates were received. In 2013, 25 isolates were received from potato, and 43 from tomato. In all years, most isolates came from England and Wales with very few from Scotland. The geographic distribution of isolates was reasonably even (*Fig. 1*). The geographic distribution of isolate origins in the 2013 tomato- and potato-hosted samples was similar (*Fig. 1c* and *1d*).

In addition to the samples collected as part of this investigation, the following reference isolates of known clonal lineage were included: 2010_8106A (23_A1), 2006_3928A (13_A2), 2006_3984C (1_A1), 2006_3888A (2_A1), and 2006_4232E (8_A1). Reference isolates were supplied by Dr David Cooke from the collection held at the James Hutton Institute, Invergowrie, Dundee, UK.

Reference isolate genotypes as determined in the present study were compared with published genotypes in order to standardise allele calling. For comparative purposes survey data from commercial potato crops for the same period, collected as part of national *P. infestans* surveys sponsored by the Potato Council, were used in the analyses (D.E.L. Cooke, James Hutton Institute, unpublished data).

DNA Extraction

Approximately 100 mg of mycelium scraped from the surface of an agar plate (or alternatively approximately 100 mg of infected plant material) was placed in a sterile collection tube and freeze-dried. DNA extraction was carried out using DNEasy Plant Mini Kits (Qiagen) according to the manufacturer's instructions.

PCR Amplification

PCR was carried out using Qiagen Multiplex PCR kits (Qiagen) with primer pairs for 12 SSR loci described by Li *et al.* (2013). Their protocol was modified to use WellRED dyes (Beckman Coulter) in two six-plex panels rather than one twelve-plex panel (*Supplementary Data 1*). The final reaction concentration of primer pair SSR4 was increased from 0.05 µM to 0.1 µM (each primer). PCR was carried out in a total volume of 12.5 µl using a PTC-100 Thermocycler (MJ Research). The reaction mixture consisted of: 6.25 µL Qiagen Multiplex PCR Master Mix (Qiagen), 0.3125 µL each primer (*Supplementary Data 1*), 1.5 µL template DNA (6 ng μL^{-1}) and 1 µL water. PCR conditions were as follows: 95 $^{\circ}$C for 15 minutes, followed by 30 cycles of 95 $^{\circ}$C for 20 s, 58 $^{\circ}$C for 90s, and 72 $^{\circ}$C for 60 s, and a final extension at 72 $^{\circ}$C for 20 minutes. PCR amplification and fragment sizing were carried out at least twice for all samples as a check against failed amplification or fragment sizing errors.

Fragment Sizing

Fragment sizing was carried out using a CEQ 8000 genetic analysis platform (Beckman Coulter), according to the manufacturer's instructions. The samples were run using the CEQ 8000 *Frag-3* programme, which consisted of a 30 second injection at 2 kV and a 35 minute capillary run at 6 kV, 50 $^{\circ}$C. Alleles were called manually using the nomenclature described by Li *et al.* (2013). The fragments produced by some primer pairs were consistently larger with the present protocol than sizes published by Li *et al.* (2013). The size of any deviation (0 - 24 bp according to locus) was established

by comparing the fragment sizes obtained using the reference samples with published fingerprints and the appropriate correction made when recording sample fragment sizes (*Supplementary Data 1*).

Datasets

Three datasets were used for different parts of the analysis:

- The *Full Dataset* was a mixed-ploidy dataset composed of all samples collected in this study.
- The *Clone-Corrected Dataset* was derived from the *Full Dataset.* Where multiple isolates had identical SSR genotypes, only one was retained (generally, the first isolate representative of a genotype to be received was used). However, in 2013, in instances where a SSR genotype was present in both the potato- and tomato-hosted sample, an isolate from each host population was retained.
- *Reference* genotypes of published named clonal lineages (Li *et al.* 2013) were included for comparison in some analyses.
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Assignment of Clonal Lineages

The *Full Dataset* plus 96 *Reference* genotypes were used to establish whether or not samples collected as part of the present study belonged to known clonal lineages. Inter-individual "Bruvo" distances (Bruvo *et al.* 2004) between all genotypes were calculated using the *meandistance.matrix* function of the package *Polysat* (Clark & Jasieniuk 2011) on the R statistics platform (R Core Team 2014). The resulting distance-matrix served as the input to the *assignClones* function in *Polysat,* in order to group the study samples with the 96 *Reference* genotypes. The grouping threshold was set at 0.15 because this was found to be the level at which the 96 *Reference* genotypes would group together within their designated clonal lineages without grouping with *Reference* isolates from other clonal lineages. Additionally, the *Phytophthora*-ID 2.0 website (Grunwald *et al.* 2014) was used to identify clonal lineages.

Detection of Underlying Genetic Structure

Shannon-Wiener and Simpson's diversity indices were calculated for each sample population. The unique isolates were treated as a single, homogeneous category and similarly, the rarest unidentified clonal lineages (New-2, New-5 and New-6) were combined into a single category. Fisher's exact test was used to test for significant differences between populations.

Principal Coordinates Analysis was carried out using the *cmdscale* function of the *stats* package in R (R Core Team 2014) to generate two principal components from the Bruvo distance table produced from the *Full Dataset.*

The *Clone Corrected Dataset* served as the input for an Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) using the R package *poppr* (Kamvar *et al.* 2014) in order to determine the proportion of genetic variability between and within populations.

Results

Population Composition

Genotypes were obtained from 119 isolates (Supplementary Data 2). Four known clonal lineages (13_A2, 6_A1, 8_A1, 23_A1 and 1_A1) were identified amongst the *P. infestans* isolates collected from gardens (*Fig. 2*). Seven "unknown" isolate groups were present (Bruvo distance <0.15) that could not be assigned to clonal lineages identified in any of the databases available (D.E.L. Cooke, James Hutton Institute, unpublished data; Grunwald *et al.* 2014; Li *et al.* 2013). Among isolates collected from gardens, 36% were unique genotypes that did not group with any other isolates and are identified here as "Unique" (*Fig. 2*).

The *P. infestans* population on commercial potato crops was dominated by the 13_A2 and 6_A1 clonal lineages during the period of this study, with these clonal lineages together making up 88%, 86% and 87% of the sample in 2011, 2012 and 2013 respectively (*Table 1*) (D.E.L. Cooke, James Hutton Institute, unpublished data). In the tomato-hosted samples from gardens for 2011, 2012 and 2013, the percentages of 13_A2 and 6_A1 together were 33%, 11% and 25%, respectively (*Fig. 2a-c*). The 2013 potato sample from gardens contained 48% 13_A2 and 6_A1 isolates (*Fig. 2d*). Isolate 23_A1 made up 20% and 14% of the samples collected from tomato in 2011 and 2012 respectively (*Fig. 2a* and *2b*), compared to a single isolate (0.23 %) in 2011 and four isolates (0.56 %) in 2012, in the sample from commercial potato crops (D.E.L. Cooke, James Hutton Institute, unpublished data). In 2013, 23_A1 was absent from both garden samples (*Fig. 2c* and *2d*) and from the commercial potato sample (D.E.L. Cooke, James Hutton Institute, unpublished data). In all years, garden samples contained a higher proportion of "Unique" isolates compared to the corresponding sample from commercial potato crops (*Table 1*), with such isolates making up 36% of the garden sample (mean of all years, both hosts), compared to 7% of the commercial potato sample (mean of all years).

Highly significant ($p<0.001$) differences were found between the distributions of clonal lineages from commercial crop populations (D.E.L. Cooke, James Hutton Institute, unpublished data) and garden populations in all years (*Table 2*). Despite the presence of different clonal lineages in the potato- and tomato-hosted populations collected from gardens in 2013 (*Figs. 2c* and *2d*), a Chi-squared test for difference in lineage frequency between the two populations did not indicate a significant difference ($p=0.107$).

Underlying Genetic Structure of Garden Populations

Principal Coordinates Analysis did not reveal clustering of isolates by sample population (*Fig. 3a*) although clustering by clonal lineage was clear among the four garden populations (*Fig. 3b*).

AMOVA (*Table 2*) showed that 92.4% of the variance across all populations was between samples within populations, and only 7.6% was between populations, with host species accounting for just 2.6% of this. Whilst the proportion of between-host variance was small, it was statistically significant ($p=0.028$).

Discussion

This study using SSR markers found little evidence of host-specialisation, despite revealing a broad palette of genetic variation in *P. infestans* populations from British gardens. The significant 2.6% genetic variation detected in 2013 suggests that there is only a small effect of host species on intra-lineage *P. infestans* variability in Great Britain. This like-for-like comparison between tomato- and potato-hosted samples from gardens was only made for one year, yet over three years isolates on tomato from gardens could not be distinguished as distinct groups of lineages when compared with isolates from commercial potato crops.

The higher diversity indices for the garden derived samples and lower proportion of the 13_A2 and 6_A1 clonal lineages in favour of rare and "Unique" genotypes, together, suggest a more varied and possibly more dynamic *P. infestans* population in gardens than in commercial potato crops. The analysis may have underestimated the diversity, particularly of the garden populations, because dissimilar genotypes were combined in the "Unique" category..

Where previous studies have examined clonal lineage using molecular marker systems (SSR or RFLP) they have generally found a clear distinction between the lineage frequencies in tomato- and potato-hosted populations (Danies *et al.* 2012; Garry *et al.* 2005; Knapova & Gisi 2002; Lebreton & Andrivon 1998; Oyarzun *et al.* 1998; Peters *et al.* 2014; Wangsomboondee *et al.* 2002). Several studies using molecular marker systems have found higher diversity on tomato (Garry *et al.* 2005; Lebreton & Andrivon 1998; Wangsomboondee *et al.* 2002), whilst others have found higher diversity on potato (Danies *et al.* 2012), or a similar diversity on both hosts (Oyarzun *et al.* 1998; Peters *et al.* 2014). In the case of the present SSR study, Shannon diversity of potato- and tomato-hosted populations of *P. infestans* from comparable (garden) settings was similar, further suggesting that these populations are not host-specialised in Great Britain.

Knapova & Gisi (2002) found conflicting results with two marker systems, with higher diversity among the potato isolates using AFLP markers, but higher diversity among tomato isolates when using SSR markers. However, the study used only two SSR markers, and other studies using larger numbers of SSR markers found that SSRs were consistent with other neutral markers (Fry *et al.* 2013; Danies *et al.* 2012). It is noteworthy that many previous studies were conducted in tropical countries where a number of hosts may be present year-round (see below). In the case of Garry *et al.* (2005), Peters *et al.* (2014) and Wangsomboondee *et al.* (2002) tomato and potato samples often came from different geographical regions, weakening any conclusion as to the effect of host on sample composition. In the present study, both potato and tomato samples were collected from throughout Great Britain (*Fig. 1c* & *1d*), and it is unlikely that there was any association between host species and geographic location. It may be that within the clonal lineages present in Great Britain, differences in aggressiveness or virulence exist, as in other countries (Danies *et al.* 2012; Delgado *et al.* 2013; Fry *et al.* 2013; Garry *et al.* 2005; Knapova and Gisi 2002; Lebreton *et al.* 1999; Oyarzun *et al.* 1998). There is scope to examine the aggressiveness and virulence of tomato- and potato-hosted *P. infestans* isolates collected in Great Britain against tomato and potato differentials carrying a variety of resistance genes.

There may be a biological reason for a lack of differentiation between tomato- and potato-hosted *P. infestans* populations in Great Britain. The British climate generally precludes *P. infestans* from overwintering on any host other than potato. Additionally, in contrast to the situation in many other European countries, oospore-mediated infections in commercial plantings appear to be rare in Great Britain (Cooke *et al.* 2014; Collins, 2013; Lees *et al.* 2012)). Therefore, in order to persist from year to year, *P. infestans* lineages must be capable of infecting potato, the only common overwintering host. A mirror situation was suggested by Le *et al.* (2008) as an explanation for the apparent absence

of host specialisation in Vietnam, where cropping cycles mean that tomato is the only available host for part of the year. However, it is notable that clear evidence of host specialisation exists in France and Switzerland (Knapova & Gisi 2002; Lebreton & Andrivon 1999) and in Canada and the north of the USA (Danies *et al.* 2012; Fry *et al.* 2013; Peters *et al.* 2014) where a similar growing situation to Great Britain exists, so the lack of continuous presence of both hosts does not appear to preclude specialisation. It is unclear if and how SSR or RFLP genotype relates to host preference, and more work is needed to ascertain the virulence and aggressiveness of *P. infestans* isolates to both hosts in order to explain the lack of apparent host-specialisation in Great Britain in contrast to the clear preference of some lineages for particular hosts in the USA, Canada, and other temperate regions.

The greater diversity found in gardens in Great Britain is in agreement with findings of Drenth *et al.* (1993b), who surveyed Dutch *P. infestans* populations and found that the A2 mating type was more common in allotment-derived samples (53%) than in those from commercial fields (12%) over the same period. Drenth *et al.* (1993a) also found greater RFLP genotype diversity in samples collected from allotments and community gardens than commercial potato fields in the Netherlands.

Possible explanations for higher diversity of *P. infestans* genotypes in gardens and allotments may include the fact that fungicides are less often used by gardeners than by commercial farmers, and this may allow fungicide-susceptible *P. infestans* genotypes to flourish in these settings. Furthermore, a wider range of varieties are grown in gardens than in commercial systems, including many "heritage" varieties developed before blight-resistance was a common breeding objective. Gardeners may therefore grow crops of susceptible potato varieties unprotected by fungicide sprays, again providing an environment in which less virulent *P. infestans* genotypes can flourish in addition to those which infect commercial crops. Infected potato seed tubers from a larger number of different sources may come together in allotment sites, facilitating the recombination of *P. infestans* genotypes through matings. This could explain the high proportion of unique isolates encountered in these settings. Finally, biosecurity measures such as removal of all "volunteer" tubers and proper disposal of outgrades are likely to be less consistent in garden and allotment settings, reducing the rate of stochastic extinction of genotypes from one season to the next.

The particular importance of tomato as a reservoir of *P. infestans* diversity in Great Britain remains unclear. This study demonstrates that *P. infestans* populations from gardens are particularly diverse, and contain a large proportion of unique genotypes possibly originating from matings. This work

highlights the need to continue efforts to educate and inform gardeners of the importance of late-blight prevention and control measures. It also emphasises the need to develop potato and tomato varieties with better late-blight resistance and promote them to gardeners.

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Figure legends

Figure 1: Origins of isolates making up the 2011 tomato sample (**1a**), 2012 tomato sample (**1b**), 2013 potato sample (**1c**) and 2013 tomato sample (**1d**). Not shown on maps are: Additional isolates were sent to the authors from unrecorded locations in Great Britain. There were four such additional isolates in 2011, four in 2012, one in 2013 (potato) and one in 2013 (tomato). sent to the authors from unrecorded locations in Great Britain; also oOne isolate was sent from Jersey in 2012 (not shown).

Figure 2: The distribution of clonal lineages within *P. infestans* samples collected from British gardens and allotments from tomato hosts (**2a-2c**) and potatoes (**2d**) collected for this study. Sample size is indicated in parentheses in the title of each graph. Segments are shaded by *P. infestans* clonal lineage. Fisher's exact test indicates significantly ($p<0.001$) different distributions for all comparisons between study samples **2a**-**2d** and the corresponding sample collected from commercial potato crops in each year (D.E.L. Cooke, James Hutton Institute, unpublished data, not shown) but not between the 2013 tomato and potato samples from gardens (**2c** and **2d)** where $p=0.107$.

Figure 3: Plots of the first two principal coordinates resulting from classical multidimensional scaling of inter-isolate genetic distances (Bruvo) calculated from combined data of 12 microsatellite markers tested on *P. infestans*blight isolates from gardens and allotments for with symbols showing each population sampled (*2a-d3a*) and and for combined data of all populations sampled, with points indicating clonal lineage identified (*2e3b*). "Other Genotypes" includes all Unique isolates and less common named clonal lineages.

Figure 4: Markov Chain Monte Carlo clustering for K=3 clusters of blight isolates, (a) grouped by sample population showing no clear association between clusters and sample populations and (b) grouped by clonal lineage showing association with cluster membership. Each isolate is represented by a vertical bar and the proportion (%) of identity to each cluster is represented by dark grey, light grey and white bars. Optimal clustering of individuals was obtained using CLUMPP (Jakobsson, Rosenberg 2007) from 20 STRUCTURE runs of 1,000,000 iterations following a 100,000 iteration burn-in period

Supplementary Data 1 – Multiplex PCR Setup: The final concentrations, WellRED dye labels, and panel groupings of the 12 microsatellite primers used in this study (after Li *et al.* (2013)). The concentration of primer SSR4-F was increased from 0.05 to 0.1 μM. Fragments detected in this study were generally larger than those published in (Li *et al.* 2013), and the deviation from the original size is indicated. The fragment sizes remained consistent over multiple PCR and fragment sizing runs. Primer stocks were prepared at the concentrations indicated so that the same volume of each could be added to the mastermix whilst retaining the desired primer ratio.

Supplementary Data 2 – Isolate SSR Fingerprints: SSR Genotypes of *P. infestans* isolates used in this study, along with the latitude and longitude of the GB post-code in which the sample was collected, and other sample metadata (where available). Some location data is missing, but all samples were collected within Great Britain. The clonal lineages indicated are those assigned in this study. Sample IDs were assigned by the authors for our own use and do not relate to any wider nomenclature system.

Table 1: Diversity indices (Shannon-Wiener and Simpson) of the garden derived samples collected for this study and samples mostly taken from commercial crops by the James Hutton Institute (D.E.L. Cooke, James Hutton Institute, unpublished data). N indicates the number of isolates in the sample. The percentages in each population are given for the two most common clonal lineages (13_A2 and 6_A1) and "Unique" isolates. Within each year, samples with different letters in the Group column were significantly different ($p < 0.001$).

Setting	Year	Population Host	N	Shannon-Wiener	Simpson	% 13_A2 and 6_A1	% Unique	Group
Garden	2011	Tomato	15	1.40	0.28	33	40	a
Commercial	2011	Mainly Potato	436	0.74	0.66	88	7	b
Garden	2012	Tomato	36	1.75	0.22	11	39	a
Commercial	2012	Mainly Potato	716	1.08	0.45	86	7	b
Garden	2013	Tomato	43	1.81	0.21	25	36	a
Garden	2013	Potato	25	1.70	0.21	48	28	a
Commercial	2013	Mainly Potato	219	1.05	0.41	87	8	b
Mean Garden	All	Both hosts	119	2.00	0.19	27	36	a
Mean Commercial	All	Mainly Potato	1371	1.04	0.46	87	7	b

Table 2: Summary of the results of Analysis of Molecular Variance (AMOVA) conducted on the *clone-corrected dataset,* indicating the percentage of genetic variation attributable to population level differences between the study years 2011-2013, between potato- and tomato-hosted populations in 2013, and between individuals.

Source of Variation	% of Total	Phi
Between Years	5.0	0.076
Between Hosts in 2013	2.6	0.028*
Between Individuals Within Populations	92.4	0.050*
Total	100.0