

Does Phytophthora infestans exhibit host specialisation on tomato in Great Britain?

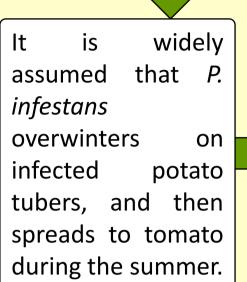
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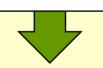
Introduction

In Britain, P. infestans infects potato (Solanum tuberosum) and tomato (S. esculentum).



The British *P. infestans* population potato comprises many genetically pathotypically distinct clones of both A1 and A2 mating types (Cooke et al. 2007).

Various forms of host specialisation observed worldwide (Goodwin et al. 1992; Oyarzun et Suassuna, & Mizubuti, 2004), but empirical studies have been carried out in Britain.



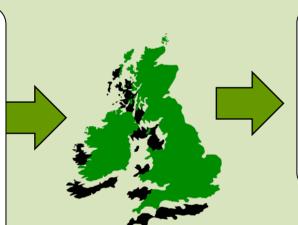
It was hypothesised that the summer tomato-host P. infestans population would comprise of a subset of the potato-host population, but may have a different frequency distribution of genotypes.

Methods

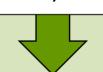
Tomato-derived *P. infestans* samples were collected from throughout Great Britain by appealing to readers of Kitchen magazine Garden members of the charity Garden Organic for samples.







using Qiagen DNeasy Plant Mini extraction kits (Qiagen no. 69106).



genotyping

was

conducted with eleven dye-labelled SSR markers (adapted from Li et al. 2013) amplified in two The SSR profiles were compared with published SSR multiplex PCR reactions. profiles of frequent P. infestans genotypes (Li et al., Fragment analysis was 2013; Cooke et al., 2011). The R applications carried out using POLYSAT and APE (R Project, 2013) were used to Beckman-Coulter CEQ8000 calculate Bruvo Distances (Bruvo et al., 2004) DNA analyser (Fig 1). between genotypes and to cluster similar genotypes.

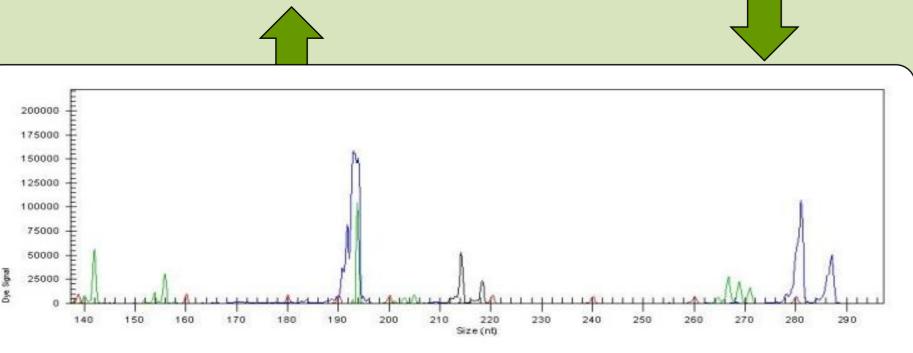


Fig 1 – Example data output from CEQ8000.

N-J Dendrogram showing relatedness of isolates

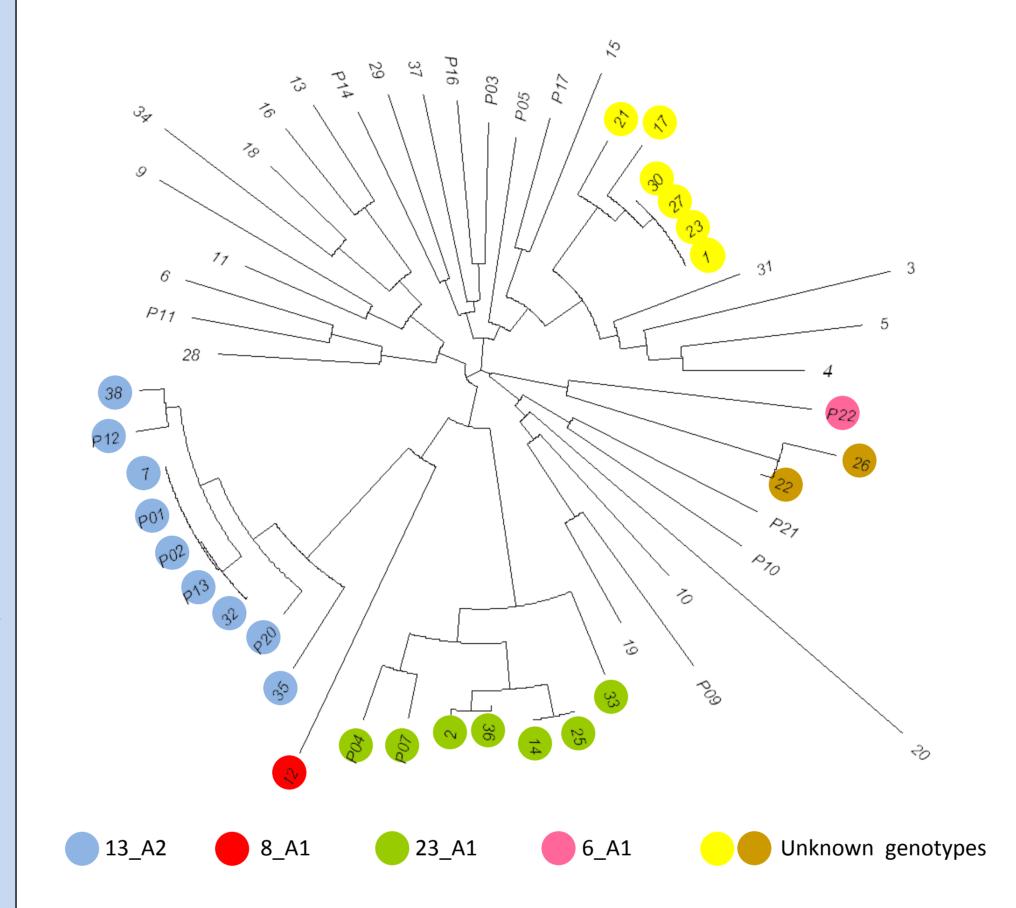


Fig. 2 – Neighbour-Joining dendrogram based on Nei's genetic distance method (Nei, 1972) showing the relatedness of all samples collected in both years. Isolates belonging to identifiable Multi Locus Genotypes (MLGs) are indicated by coloured markers. Miscellaneous isolates separated by a Bruvo distance of more than 0.1 were considered unique (no coloured marker). The 17 isolates collected in 2011 are prefixed with "P". The 36 isolates collected in 2012 labelled with unprefixed numbers.

Discussion

Different population compositions were observed between tomato and potato isolates, despite the small sample tested. Specific genotypes such as 6_A1 and 23_A1 contrasted in frequency between the two hosts, and this may be explained by differential virulence (Legard, Lee & Fry, 1995) and rate of growth and sporulation (Suassuna, Maffia & Mizubuti, 2004) on different hosts. Reasons why the numerous diverse "Misc." isolates were so much more prevalent on tomato than potato are unclear. However, it has been reported anecdotally that "Misc." isolates tend to be more common in allotments and home gardens (the source of the tomato isolates use here) than in agricultural settings (Shaw, 2013) where most of the potato isolates were derived. To date, the British tomato-hosted P. infestans population has not been well studied, and further investigation of host-specialisation promises to be an interesting area of research. Future work will attempt to address these questions, and also to investigate relationships between mating type, pathotype, genotype, and host.

DNA was obtained from 17 tomato-derived P. infestans isolates collected in 2011, and from 36 collected in 2012.

- Much genetic diversity was found between isolates, with some clustering as Multi-Locus Genotype (MLGs) (Fig. 2)
- Tomato isolates from both years appear strikingly different in composition from the national population of (mainly) potato isolates collected as part of the "Fight against Blight" (British Potato Council, 2013). The MLG composition of each population is displayed in Fig. 3.
- 6_A1 genotypes make up 80% and 60% of all potato isolates nationally in 2011 and 2012, but only 6% of tomato isolates in 2011 and none in 2012 (Fig. 3).
- In both years, the composition of tomato population differed significantly from that of the national (potato) population (2011: $\chi^2 = 50.04$, p <0.001; 2012: $\chi^2 =$ 101.6, p < 0.001) confirming that the tomato and potato populations are very different. (8_A1 and 23_A1 counts were integrated into the "Misc." counts in order to satisfy minimum expected value requirements of the Chi-squared test.)

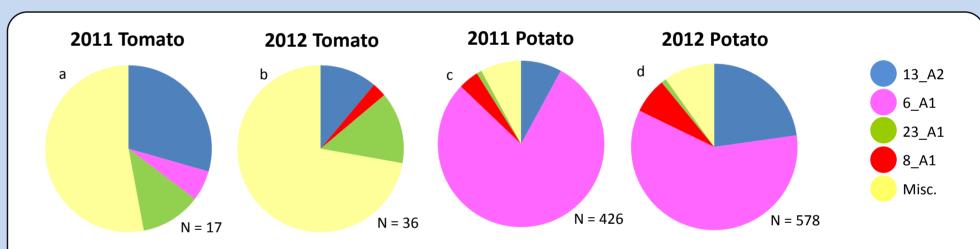


Fig. 3 – Genotypic composition of tomato-host *P. infestans* sample in 2011 (a) and 2012 (b) and the composition of the national samples (mostly potato-host) held by the James Hutton Institute (c, d) (Cooke, 2013). Chi-square tests were carried out after pooling 23_A1, 8_A1, and Misc. in order to achieve adequate minimum expected values. 2011 χ^2 = 50.04, p <0.001. 2012 χ^2 = 101.6, p <0.001

References

Results

British Potato Council, 2013. Fight against Blight campaign. http://www.potato.org.uk/node/910

Bruvo, N.K. Michiels, T.G. D'Souza, H. Schulenburg. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. *Mol. Ecol.*, 13, pp. 2101–2106.

Cooke D.E.L, Lees A.K., Shaw D.S., Taylor M, Prentice M.W.C., Bradshaw N.J. and Bain R.A. 2007. Survey of GB blight populations. PPO-Special Report no. 12, 145-151

Cooke, D.E.L., 2013. Pers comm.

Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. & Fry, W.E. 1992. Clonal Diversity and Genetic Differentiation of Phytophthorainfestans Populations in Northern and Central Mexico. Phytopathology, vol. 82, no. 9, pp. 955-961.

Legard, D., Lee, T.Y. & Fry, W.E. 1995, "Pathogenic specialization in *Phytophthora infestans*: Aggressiveness on tomato", *Phytopathology*, vol. 85, no. 11, pp. 1356-1361.

Li, Y., Cooke, D.E.L., van der Lee, T., Jacobsen, E., 2012. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen Phytophthora infestans. Journal of Microbiological Methods 92, 316-322.

R Project, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-

Nei, M., 1972. Genetic Distance between Populations. The American Naturalist, Vol. 106, No. 949, pp. 283-292. Published by: The University of Chicago Press for The American Society of Naturalists.

Oyarzun, P.J., Pozo, A., Ordonez, M.E., Doucett, K. & Forbes, G.A. 1998. Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador, Phytopathology, vol. 88, no. 3, pp. 265-271.

Shaw, D.S., 2013. Pers. Comm.

Suassuna, N., Maffia, L. & Mizubuti, E. 2004. Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*, *Plant* Pathology, vol. 53, no. 4, pp. 405-413.

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