

Monitoring the Danish population of potato late blight pathogen, *Phytophthora infestans* in 2011-2012 and occurrence of 13_A2

BENT J NIELSEN¹, DAVID E L COOKE², & JENS G HANSEN¹

¹Aarhus University, Department of Agroecology, Denmark

²The James Hutton Institute, Invergowrie Dundee, DD2 5DA, UK

SUMMARY

Samples of the potato late blight pathogen (*Phytophthora infestans*) were collected from different potato fields in Denmark in 2011 and 2012 and genotyped using simple sequence repeat (SSR) markers. The results showed that the Danish *P. infestans* population is highly diverse with only a few minor clones that were spread locally. The diversity in the data indicate oospore-borne inoculum is important in Danish potato crops. The only dominant genotypes, 13_A2 and dk_6, were present in both seasons with 13_A2 comprising 12% in 2011 and 25% in 2012.

KEYWORDS

Potato late blight, *Phytophthora infestans*, monitoring, Danish population, genotype 13_A2, dk_6

INTRODUCTION

Knowledge of the genetic diversity and structure of pathogen populations advances applied and fundamental science. At a practical level, it helps the industry understand sources of primary inoculum and, if combined with data on the traits of the local pathogen population (e.g. fungicide resistance) it can improve disease management decisions. Such knowledge also provides insights into the rate and mechanisms of evolutionary change within the pathogen population.

Previous surveys have indicated a diverse population across the Nordic region (Brurberg *et al.*, 2011, Sjöholm *et al.*, 2013) that suggests sexual recombination and a significant role of oospores as a source of primary inoculum (Widmark *et al.*, 2011, Yuen & Andersson, 2012). In contrast, other regions of Europe are characterised by relatively few dominant clonal lineages with a lower proportion of genetically unique recombinant isolates (e.g. Li *et al.*, 2012, Cooke *et al.*, 2012). One aggressive lineage in particular, termed 13_A2 (Cooke *et al.*, 2012), spread rapidly from 2004 onwards to become locally dominant. It is now widespread in some parts of Europe (Chmielarz *et al.*, 2013, Cooke, unpublished data) and found in China (Li *et al.*, 2013a) and India (Chowdappa *et al.*, 2012). It has not, to date, been reported in the Nordic regions. In

this study we investigated the structure of the Danish *P. infestans* population in more detail than previous studies by sampling late blight outbreaks potato crops over the 2011 and 2012 seasons. A specific emphasis in this study was to examine the causes of any incursion of the 13_A2 lineage into the Danish potato production regions.

MATERIALS AND METHODS

Late blight infected leaf samples were collected from potato fields in Denmark in early September 2011 and from July to September 2012. The sampling targeted potato fields from across different potato growing areas with one single-lesion leaflet chosen per field. Lesions were pressed onto FTA cards (Whatman) for later genotyping. Identification of genotypes using a 12-plex SSR protocol was performed according to a published protocol (Li *et al.*, 2013b). The resultant fingerprints were compared to other defined lineages in published and unpublished data held at the James Hutton Institute.

RESULTS

In 2011, a total of 74 samples were collected from 52 fields and 9 samples (12%) were of the genotype 13_A2 ("Blue 13") lineage. Most of the 13_A2 samples (6) came from North Jutland (starch potato producing area). In 2012, a total of 112 samples from 102 fields were tested and 28 samples (25%) were of the genotype 13_A2. In this season, 22 of the 28 13_A2 samples came from North Jutland and 6 samples of 13_A2 from mid-Jutland. No samples matching the "green33" lineage were found in either year. Other samples were of locally distributed clonal types or unique genotypes found only once (Fig. 1).

DISCUSSION

The results show that the Danish *P. infestans* population is highly diverse (only two clones comprised more than 3% of the population). Sub-clonal SSR variation is evident and other minor clones are mostly locally spread. The only dominant genotypes, 13_A2 and dk_6, were present in both seasons. The diversity in the data indicates that oospore-borne inoculum is important in Denmark and that some inoculum from these initial sources is propagated within crops and spread locally as it was sampled again later in the same season.

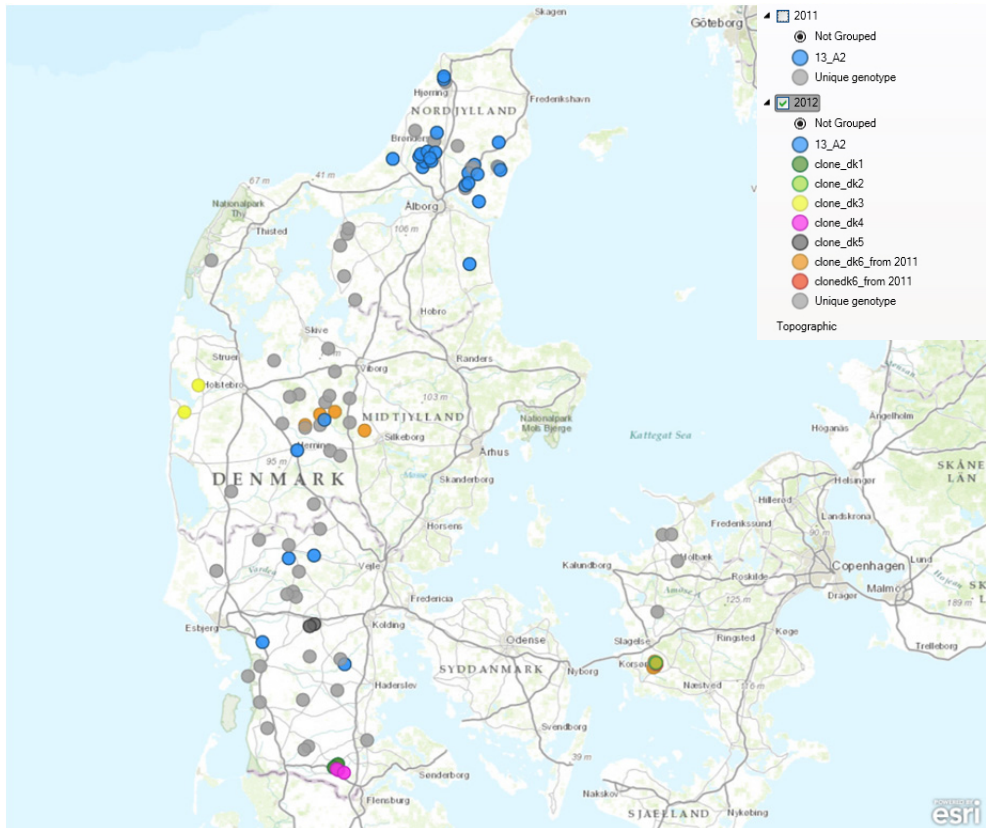


Figure 1. Distribution of *P. infestans* genotypes in Denmark 2012. Isolates were collected July – September 2012 from different potato fields and conserved on FTA cards for subsequent SSR genotyping

Most of the 13_A2 genotype samples came from North Jutland where there is a high concentration of varieties grown for starch production. In 2012, the first record of late blight infection was 3th July and over the following 2-3 weeks many fields were sprayed with metalaxyl (Ridomil Gold) curatively to control the outbreaks. The collection of isolates in the area was started from the 13th-19th of July and many isolates were of the 13_A2 genotype.

Resistance to metalaxyl has been demonstrated in the 13_A2 genotype (Gisi *et al.*, 2011, Cooke *et al.*, 2012) and the prevalence of 13_A2 in North Jutland may thus be a consequence of a selection pressure generated by curative metalaxyl usage. Prior metalaxyl testing in Denmark has shown that 5% of the *P. infestans* isolates sampled were resistant in 2003 and 25% in 2008 (based on the leaf disk method, Hannukkala, unpublished). In another study, 8% of isolates collected in 2006 to 2007 were resistant (Gisi *et al.*, 2011).

Further sampling in 2013 and a detailed analysis of SSR fingerprints, combined with spatial analysis and crop management information from three consecutive potato growing seasons will shed more light on the drivers of *P. infestans* population change in Denmark.

REFERENCES

- Brurberg, M.B., Elameen, A., Le, V.H., Naerstad, R., Hermansen, A., Lehtinen, A., Hannukkala, A., Nielsen, B., Hansen, J.G., Andersson, B., Yuen, J., 2011. Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology* 115: 335-342.
- Chmielarz, M., Sobkowiak, S., Dezbski, K., Cooke, D.E.L., Brurberg, M.B., Śliwka, J., 2013. Diversity of *Phytophthora infestans* from Poland. *Plant Pathology* Early view Doi: 10.1111/ppa.12076
- Chowdappa, P., Kumar, N.B.J., Madhura, S., Kumar, M.S.P., Myers, K.L., Fry, W.E., Squires, J.N., Cooke, D.E.L., 2012. Emergence of 13_A2 blue lineage of *Phytophthora infestans* was responsible for severe outbreaks of late blight on tomato in South-West India. *Journal of Phytopathology* 161: 49-58.
- Cooke, D.E.L., Cano, L.M., Raffaele, S., Bain, R.A., Cooke, L.R., Etherington, G.J., Deahl, K.L., Farrer, R.A., Gilroy, E.M., Goss, E.M., Grünwald, N.J., Hein, I., MacLean, D., McNicol, J.W., Randall, E., Oliva, R.F., Pel, M.A., Shaw, D.S., Squires, J.N., Taylor, M.C., Vleeshouwers, V.G.A.A., Birch, P.R.J., Lees, A.K., Kamoun, S., 2012. Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. *PLoS Pathogens* 8(10) e1002940. doi:10.1371/journal.ppat.1002940
- Li, Y., van der Lee, T.A.J., Evenhuis, A., van den Bosch, G.B.M., van Bekkum, P.J., Förch, M.G., van Gent-Pelzer, M.P.E., van Raaij, H.M.G., Jacobsen, E., Huang, S.W., Govers, F., Vleeshouwers, V.G.A.A., Kessel, G.J.T., 2012. Population dynamics of *Phytophthora infestans* in the Netherlands reveals expansion and spread of dominant clonal lineages and virulence in sexual offspring. *G3 Genes Genomes Genetics* 2: 1529-1540.
- Li, Y., van der Lee, T., Zhu, J.H., Jin, G.H., Lan, C.Z., Zhu, S.X., Zhang, R.F., Liu, B.W., Zhao, Z.J., Kessel, G., Huang, S.W., Jacobsen, E., 2013a. Population structure of *Phytophthora infestans* in China – geographic clusters and presence of the EU genotype Blue_13. *Plant Pathology* 62: 932-942.
- Li, Y., Cooke, D.E.L., van der Lee, T., Jacobsen, E., 2013b. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen *Phytophthora infestans*. *Journal of Microbiological Methods* 92: 316-322.
- Sjöholm, L., Andersson, B., Högberg, N., Widmark, A., Yuen, J., 2013. Genotypic diversity and migration patterns of *Phytophthora infestans* in the Nordic countries. *Fungal Biology* 117: 722-730.
- Widmark, A.-K., Andersson, B., Sandström, M., Yuen, J.E., 2011. Tracking *Phytophthora infestans* with SSR markers within and between seasons – a field study in Sweden. *Plant Pathology* 60: 938-945.
- Yuen, J.E., Andersson, B., 2012. What is the evidence for sexual reproduction of *Phytophthora infestans* in Europe? *Plant Pathology* 62:485-491.