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Introduction

Phytophthora ramorum is an aggressive, introduced pathogen of unknown geographical origin. Current known distribution is western North America and parts of Europe (Grünwald et al. 2012). In North America, it is known as the cause of 'sudden oak death'. In the UK it affects mainly rhododendron, but from 2009 onwards has caused heavy dieback and mortality of Japanese larch (*Larix kaempferi*).

The pathogen exists as three distinct lineages (Ivors et al. 2006) designated as NA1, NA2 and EU1 (Grünwald et al. 2009). NA1 and NA2 lineages are confined to North America and, until recently, the EU1 lineage was the only form found in Europe and caused most *P. ramorum* outbreaks on larch in the UK. However, in 2011 a fourth unique lineage was discovered, designated as EU2 (Figure 1), and limited to the Antrim Plateau, Northern Ireland and the Ayrshire/Galloway border region of south west Scotland (Van Poucke et al. 2012).

Recent studies show that the EU2 lineage is especially aggressive to larch (Harris & Webber 2013), so understanding the distribution of this new lineage and how quickly it may be spreading beyond the 2011 findings is critical to effective management of *P. ramorum*.

	EU2-specific	EU1-specific
EU1:	T A A C A G G T G T G G C -- A A A T C C A T T T T T G	T A A C T G G T G T G G C -- A A A T T C A T T T T T G
EU2:	T A A C T G G T G T G G C -- A A A T C C A T T T T T G	T A A C A G G T G T G G C -- A A A T T C A T T T T T G
NA1:	T A A C A G G T G T G G C -- A A A T T C A T T T T T G	T A A C A G G T G T G G C -- A A A T T C A T T T T T G
NA2:	T A A C A G G T G T G G C -- A A A T T C A T T T T T G	T A A C A G G T G T G G C -- A A A T T C A T T T T T G

Figure 1: Differences in *Cox1* gene sequences (hashed boxes) provided a diagnostic test to discriminate between all four lineages of *Phytophthora ramorum*, including EU1 and EU2.

Experimental approach

- A combination of microsatellite profiling and DNA sequence analysis of *Cox1* was used to assign the lineage of *P. ramorum*.
- Over 300 UK isolates of *P. ramorum* in the Forest Research Phytophthora Culture collection and accumulated between 2002-2013 were screened for lineage using microsatellite markers (Vercauteren et al. 2010).
- However, it has proved very difficult to culture *P. ramorum* from infected larch tissue, so to explore the distribution of EU1 and EU2 lineages on affected larch a new approach was needed.
- Therefore, a method was developed to determine *P. ramorum* lineage by using DNA directly extracted from samples of infected larch tissue. This new method was an extension of a diagnostic test devised by Van Poucke et al. (2012) and previously only applied to isolates.
- It was essential to 'clean' the *P. ramorum* DNA to remove inhibitors carried over from the larch tissue before screening for lineage.
- In all, 101 *P. ramorum* infected larch samples collected during 2013 from England, Scotland and Wales were tested for lineage using the new method.

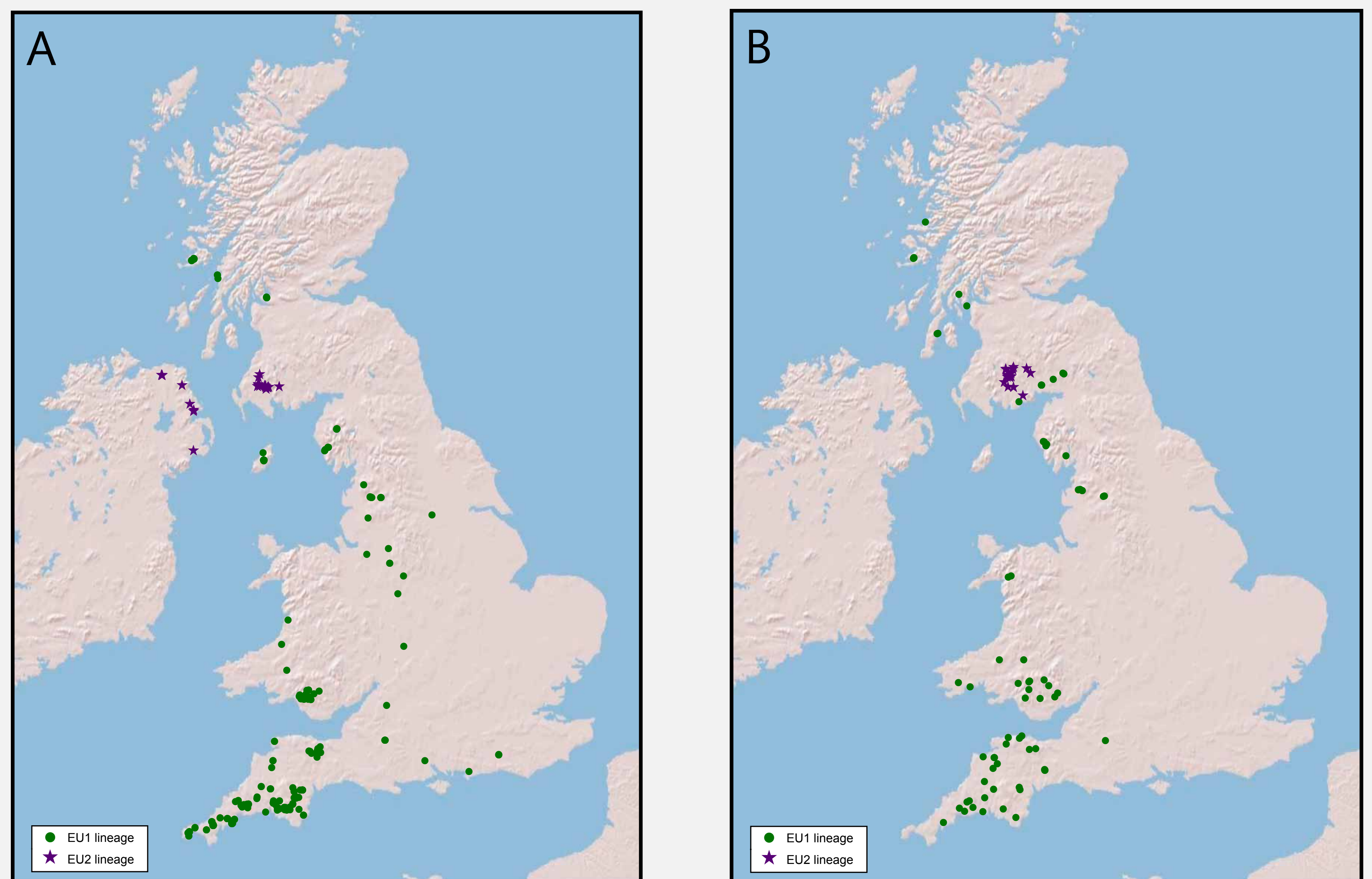


Figure 2: Distribution of EU1 and EU2 lineages in the UK based on (A) microsatellite screened isolates and (B) field samples collected in 2013.

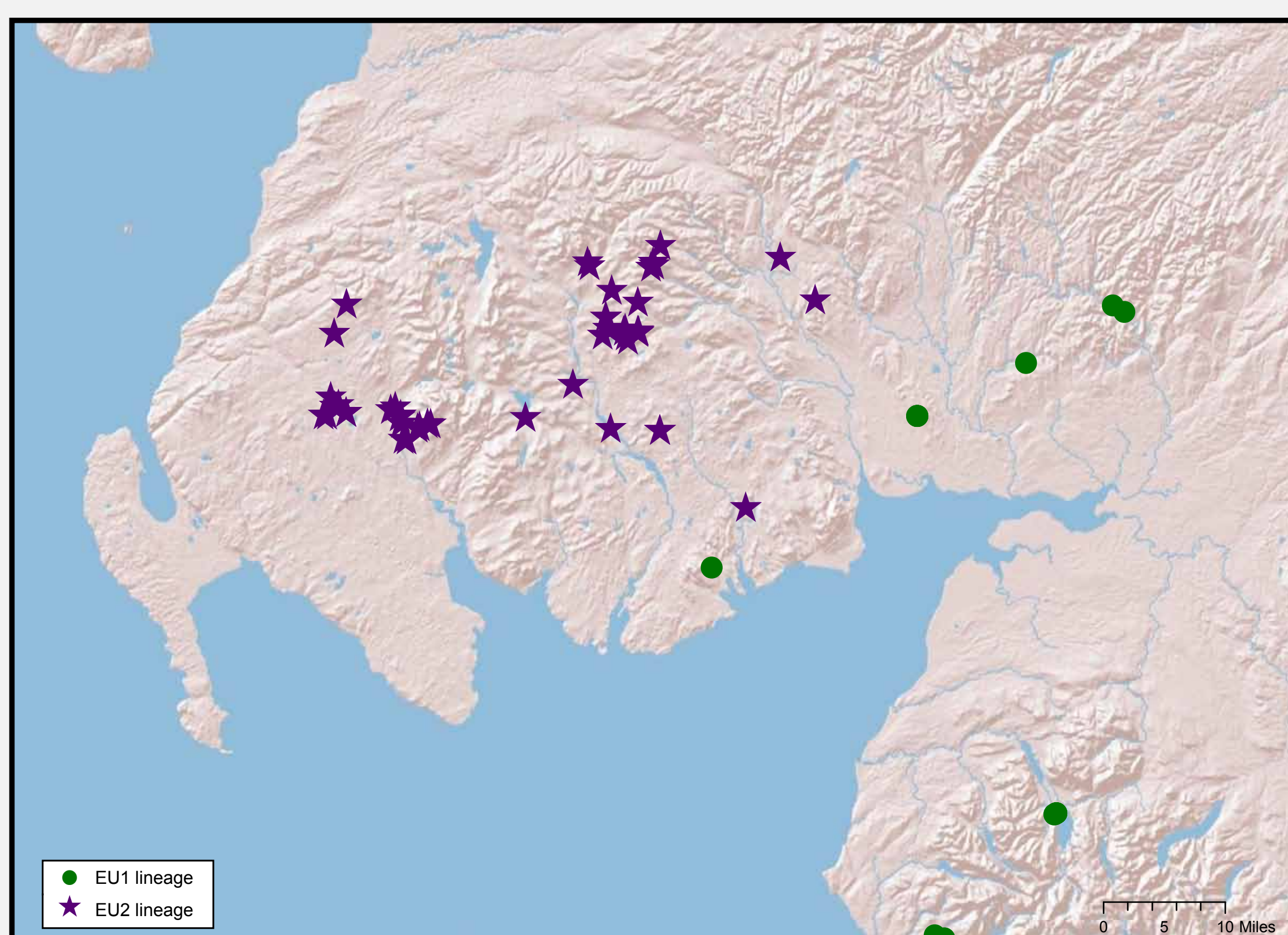


Figure 3: Current detailed distribution of EU1 and EU2 lineages in south west Scotland based on both isolates and field samples.

Results

- The earliest finding of the EU2 lineage was from County Down in Northern Ireland, dating from November 2007.
- Only the EU1 lineage was widely distributed throughout England and Wales, with no EU2 detected (Figure 2).
- The EU2 lineage of *P. ramorum* was only found in Northern Ireland and south west Scotland, as found previously with seven isolates collected in 2011 (Van Poucke et al. 2012).
- However, the EU2 was more widespread in south west Scotland than previously known. It was found further south and east, co-existing in some areas with the EU1 with both lineages of *P. ramorum* infecting larch (Figure 3).

Conclusions

- A new diagnostic method was developed to test for lineage directly in infected larch tissue.
- The EU2 remains confined to Northern Ireland and the Ayrshire/Galloway border region of south west Scotland. In adjacent locations, such as the Isle of Mull, coastal west Scotland, the Isle of Man and Cumberland and Lancashire in north west England only EU1 is present.
- Without controls the EU2 will probably spread further via movement of infected plant material, on machinery or by natural spread, for example, sporangia in wind-blown mists.
- As the EU2 lineage of *P. ramorum* appears to be adaptively different from the EU1 lineage already present in the UK (Franceschini et al. 2014) and more damaging to larch (Harris & Webber 2013) this possibility should be viewed with some concern.

References

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