

INTRODUCTION

Pseudomonas savastanoi pv. *fraxini* (Psf) is a bacterium that causes ash canker and is widespread in the UK (figure 1). With the widespread infection of ash dieback, the health of the ash tree population is decreasing. However, little is understood about the genetic diversity or variation in virulence of Psf and its interaction with ash dieback and their host. This study aims to establish the genetic diversity and population structure of Psf in the UK.

METHODS

- 1) Sampling - bark tissue was collected from healthy and diseased ash from six woodland sites in the UK
- 2) Isolation - epiphytic and endophytic bacteria were cultured on *Pseudomonas* selection media
- 3) Molecular characterisation - repetitive-element PCR (rep-PCR) was conducted for all Psf isolates (figure 2)
- 4) Statistics - distance matrix based on Jaccard's similarity index was calculated and analysed with UPGMA clustering algorithm

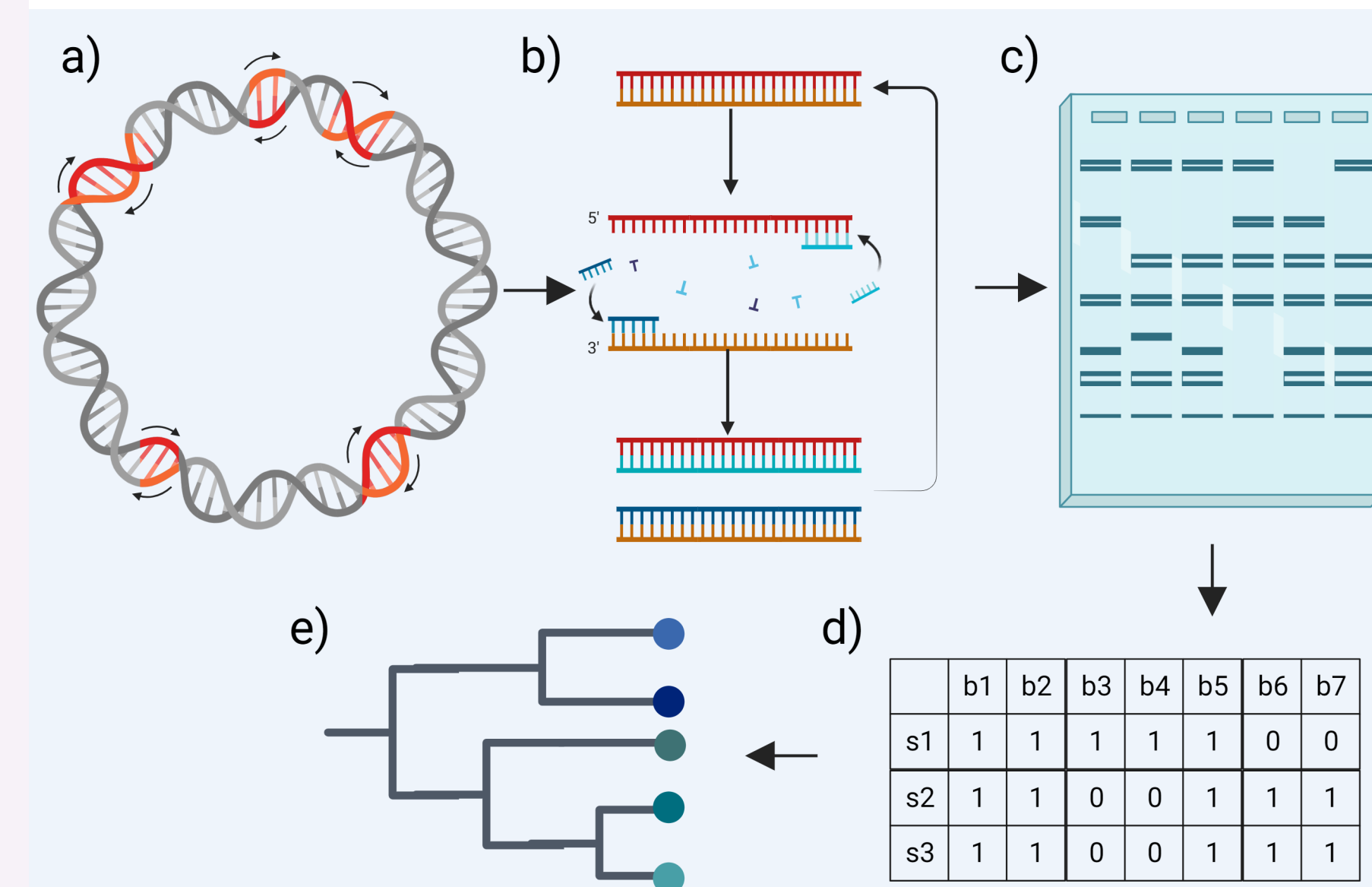


Figure 2- rep-PCR workflow. a) conserved repetitive regions dispersed throughout the bacterial genome; b) amplification of repetitive regions by PCR using primers flanking the conserved regions (rep-primers); c) gel electrophoresis separates out amplicons based on size, d) presence/absence of bands (b1..) for each sample (s1..) converted to a binary data matrix, e) hierarchical clustering calculated from a distance matrix.

RESULTS

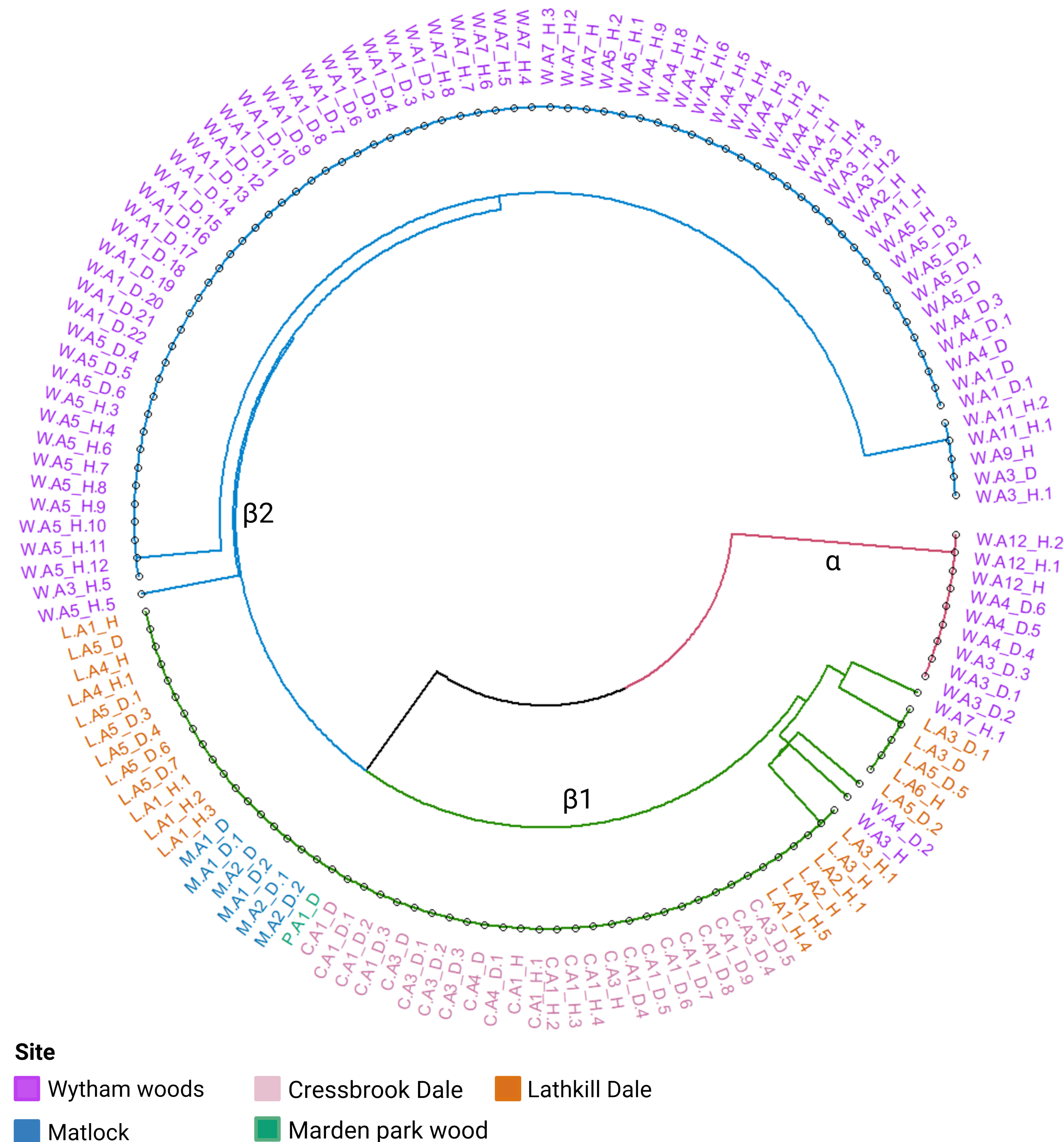


Figure 3- Genetic relatedness of 142 Psf isolates based on UPGMA analysis, obtained with rep-PCR primer sets. Sites are represented by different colour labels: purple (Wytham woods); orange (Lathkill Dale); blue (Matlock), green (Marden Park), pink (Cressbrook Dale). Trees labelled A1 to A6 are diseased, whilst A7-A12 are healthy. One supercluster is split into two sub-clusters: $\beta 1$ (green) & $\beta 2$ (blue). A third clade is labelled α (pink). The goodness-of-fit was calculated using cophenetic correlation for Dice and Jaccard's coefficients with four clustering algorithms Results showed Jaccard's index and unweighted pair group method (UPGMA) was the best model, with a goodness-of-fit of 0.936.

RESULTS

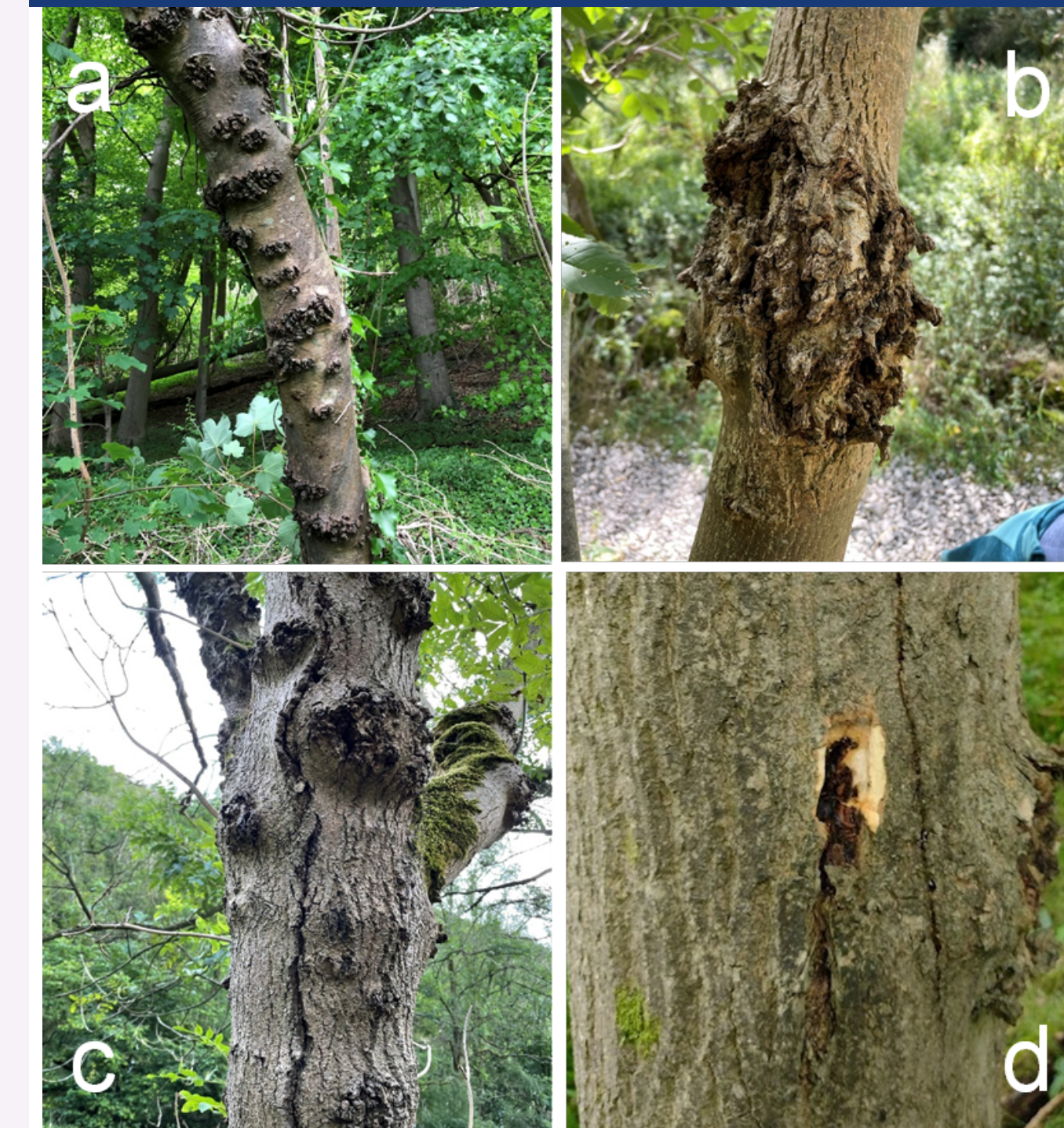


Figure 1- Different canker types formed after *Psf* infection a) letterbox cankers cover the trunk, suggesting systemic spread; b) corky outgrowth; c) vertical splits down the main stem, possibly exacerbated by frost damage; d) the inside of a bleeding lesion, secreting a brown sticky exudate, this is likely the initial stages of new infections.

Conclusions

From nine genotypes detected, two are present in 84% of strains (figure 3). Thus, *Psf* is clonal and relatively homogenous. The population of *Psf* in the UK may have evolved from a single lineage, or evolved from the same environmental strain independently multiple times.

Isolates with distinct genotypes were extracted from the same site, and even the same tree on numerous occasions. Polymorphisms in a small number of strains may be due to divergence during extensive co-evolution with ash trees. Whole genome sequencing will be conducted for all strains to further investigate this.