



New methods to detect the threat of *Phytophthora* in irrigation water



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142 species of *Phytophthora*



Air- or soil-borne but
absolute requirement for
water in all species & that
provides a means of detecting
zoospores





Challenges

- Symptoms can be non-specific
- Difficult to isolate
- Species difficult to distinguish morphologically



Rhododendron with wilting symptoms caused by *Phytophthora* root rot.





Diagnosis versus Detection

If you already have a problem

- Conventional molecular diagnostic method
 - plant material
 - DNA extraction
 - Specific PCR tests (e.g. *P. rubi*, *P. idaei*)

If you want to avoid a future problem

- Detecting unknown threat
 - Irrigation water testing
 - Planting material testing
 - Keeping out new threats – Biosecurity

Water testing can help



Phytophthora zoospore detection

Sampling water

- Irrigation water
- Water flooded through roots of pot-grown plants (factors such as timing, temperature are important)
- Rivers



Filtration

- Cellulose acetate filters
- DNA extraction
- PCR with *Phytophthora* species or genus specific primers
- Sequencing (high or low throughput)





Practical application of filtration method

- Proven detection of *P. rubi* and *P. idaei* on water flowing through coir in raspberry production systems
- Current funding to test this on large batches of plants in plant health testing stations to support plant health legislation
- Using high throughput technologies for sequencing hundreds of samples to a great depth (thousands of reads) in a single run





Environmental sampling



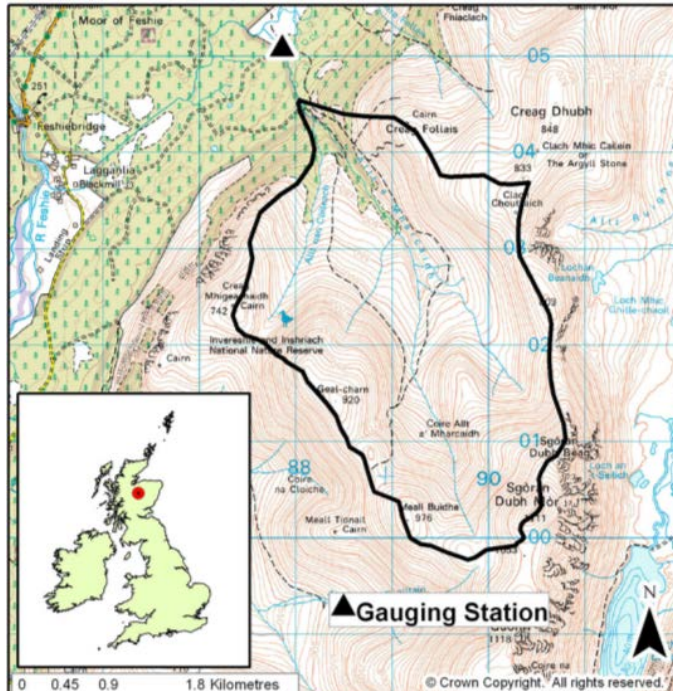
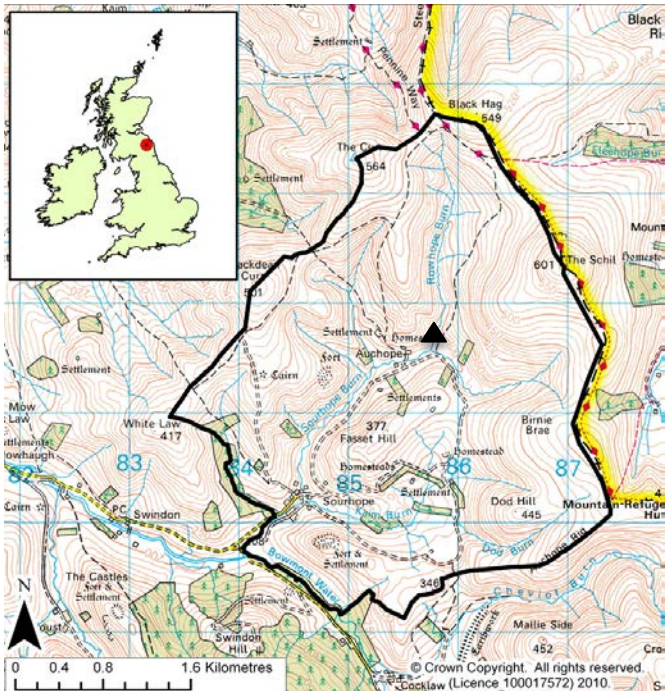
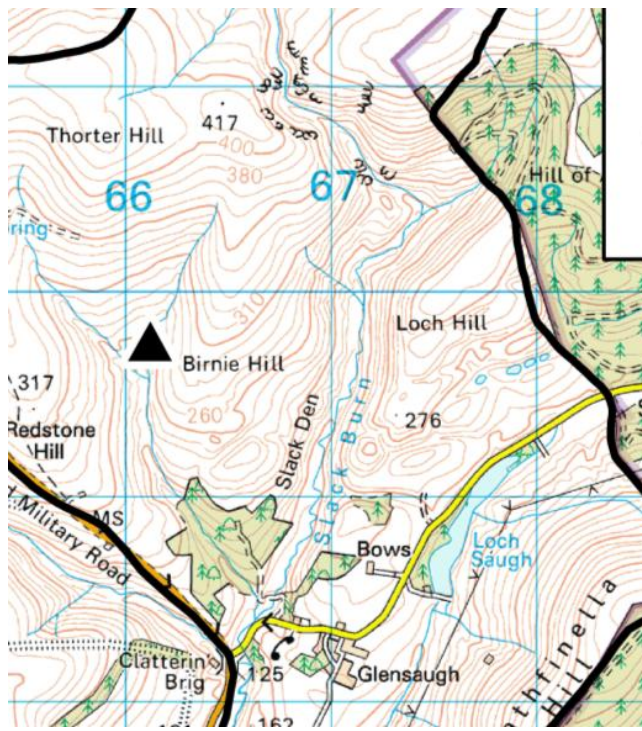
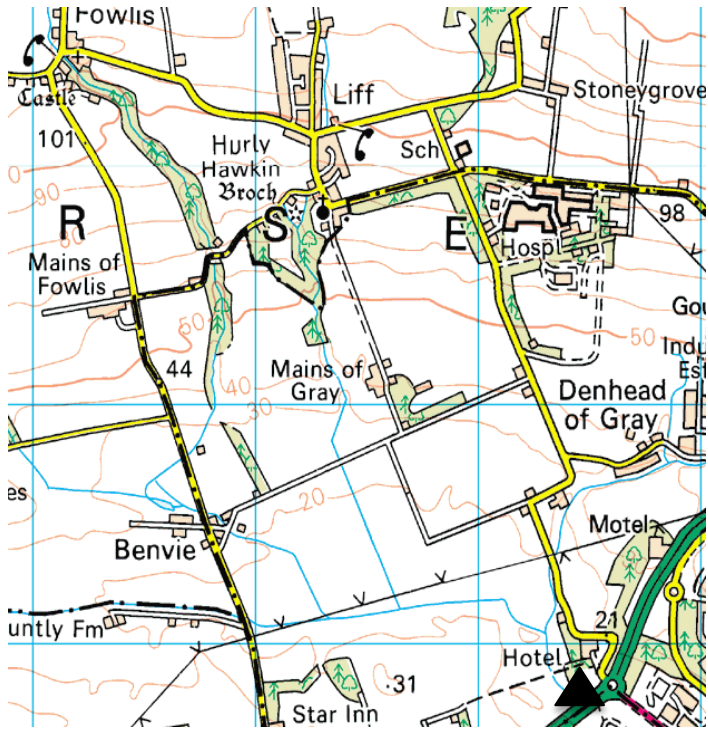
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- Four sample sites (3 = ECN)
 - Invergowrie Burn (IGB)
 - Glensaugh Burn (GSB)
 - Sourhope Burn (SRB)
 - Allt a'Mharcaidh Burn (ECN)
- Sites apparently healthy
- 10 litre water samples collected and filtered every two weeks from Dec 2011 to Feb 2014
- Filters kept cool on day of sampling and then frozen
- IGB most heavily sampled and baiting applied here only





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Metabarcoding method

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A molecular method to assess *Phytophthora* diversity in environmental samples

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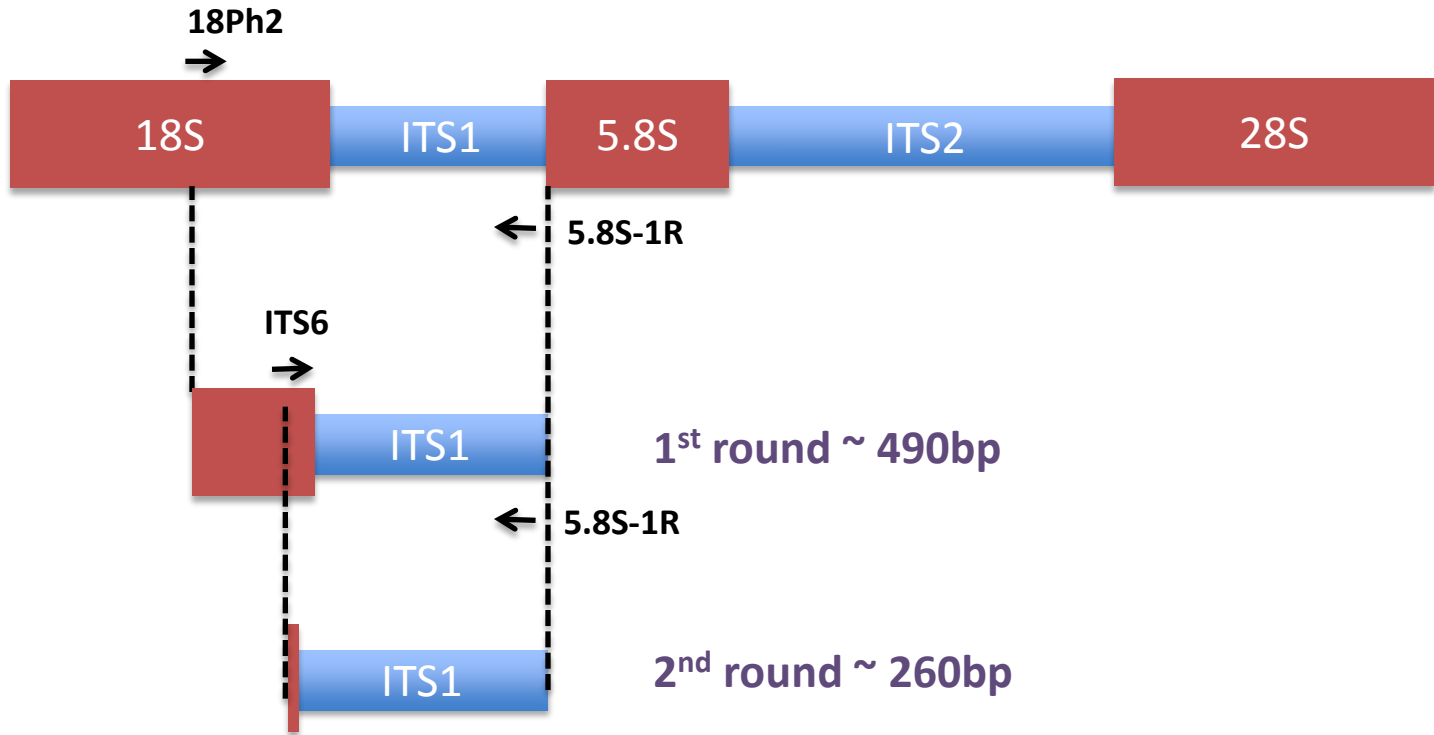
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Primer position and product size

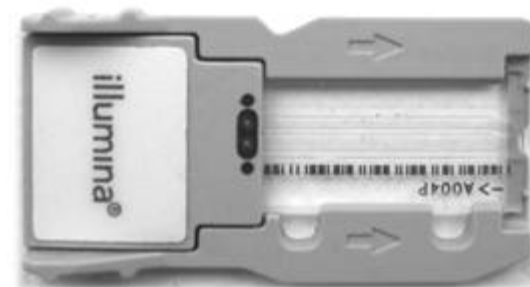


- Two rounds of semi-nested PCR required



Illumina MiSeq

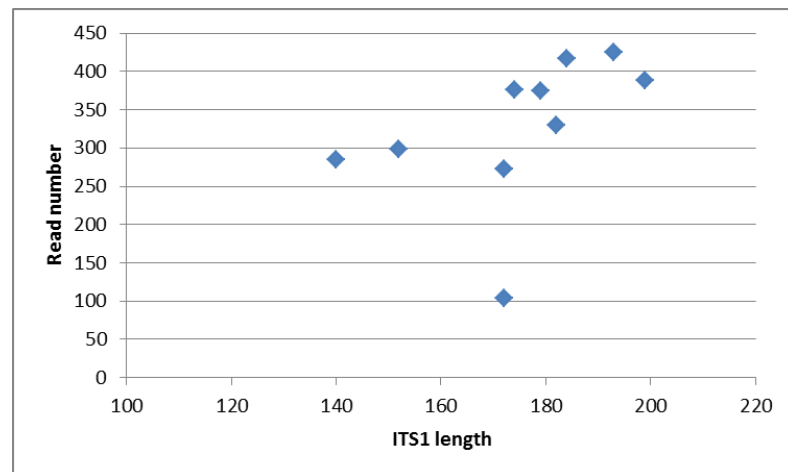
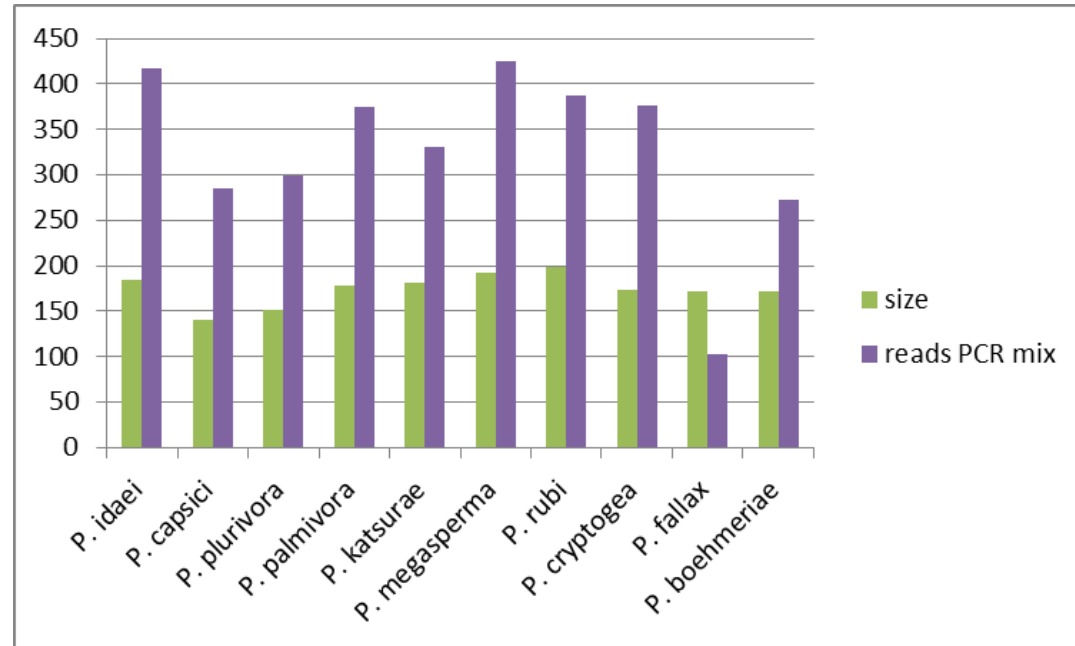
- 96 samples tagged, pooled and sequenced in a nano-cell (1 million reads)
- De-randomised – error corrected resulting in 890K reads (median 8172 per sample)
- Bioinformatics pipeline...





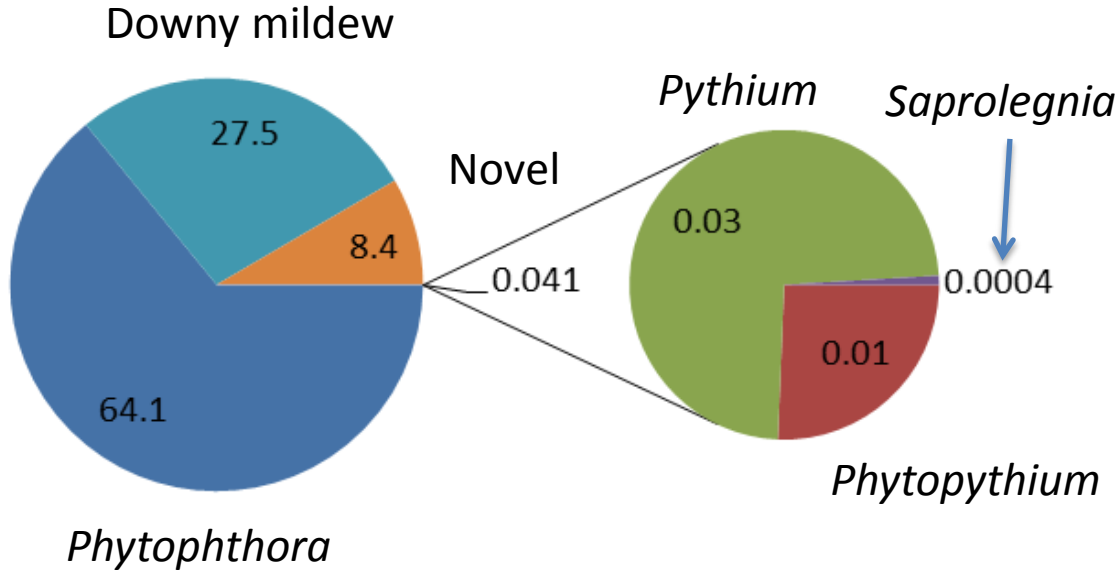
Control reaction results

- The **PCR mix** worked well
- Inverse relation to product size
- *P. fallax* fewer reads





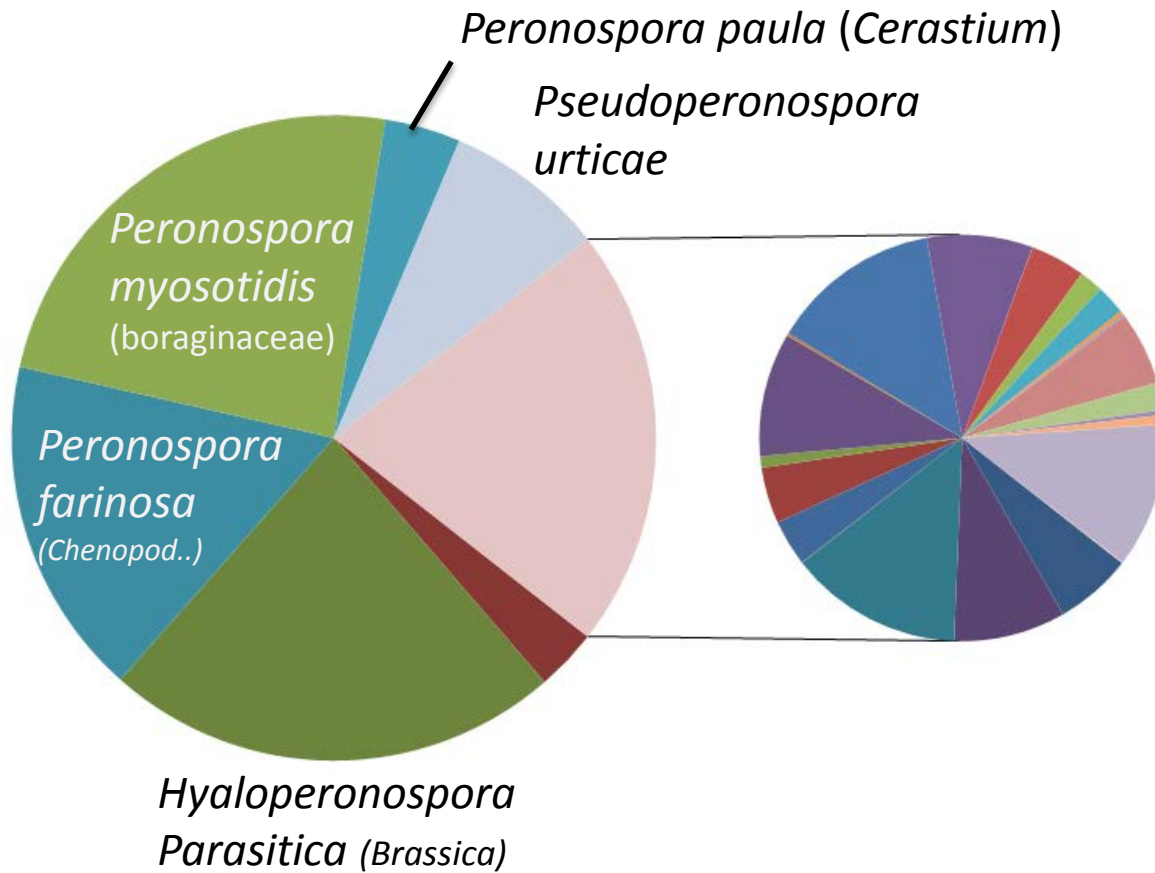
Taxonomic summary MiSeq



- Majority of reads *Phytophthora*
- Almost 30% downy mildew species
- 8 % novel – mostly new *Phytophthoras*
- Tiny proportion of other reads so primers highly specific



Downy mildew summary MiSeq

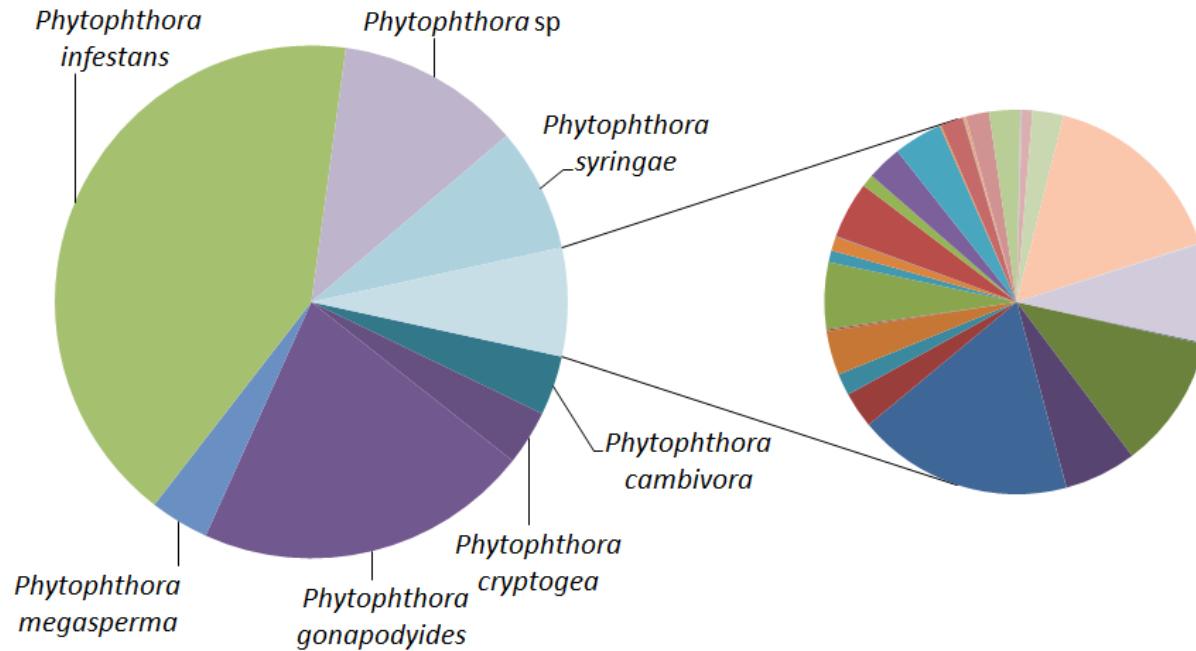


- 37 species - a lot of diversity
- 27 *Peronospora*
- 2 *Hyaloperonospora*
- 1 *Bremia* (*lactucae*)
- 3 *Pseudoperonospora*
- 4 *Plasmopora*

- Range of obligate species seems logical for plant hosts likely in these environments



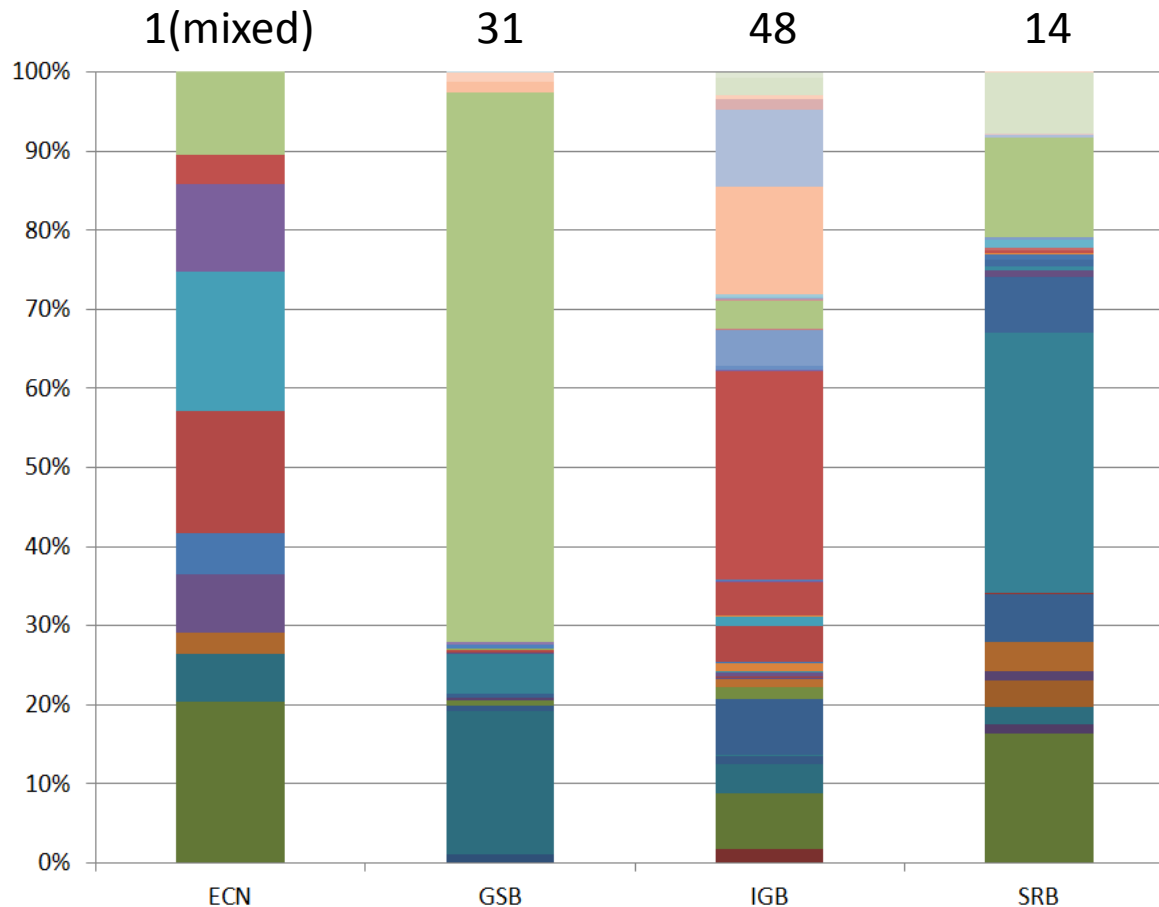
Phytophthora summary MiSeq



- 45 known species
- *P. infestans*? 85% of these in GSB and generally weaker samples
- *P. gonapodyides* most abundant phylotype



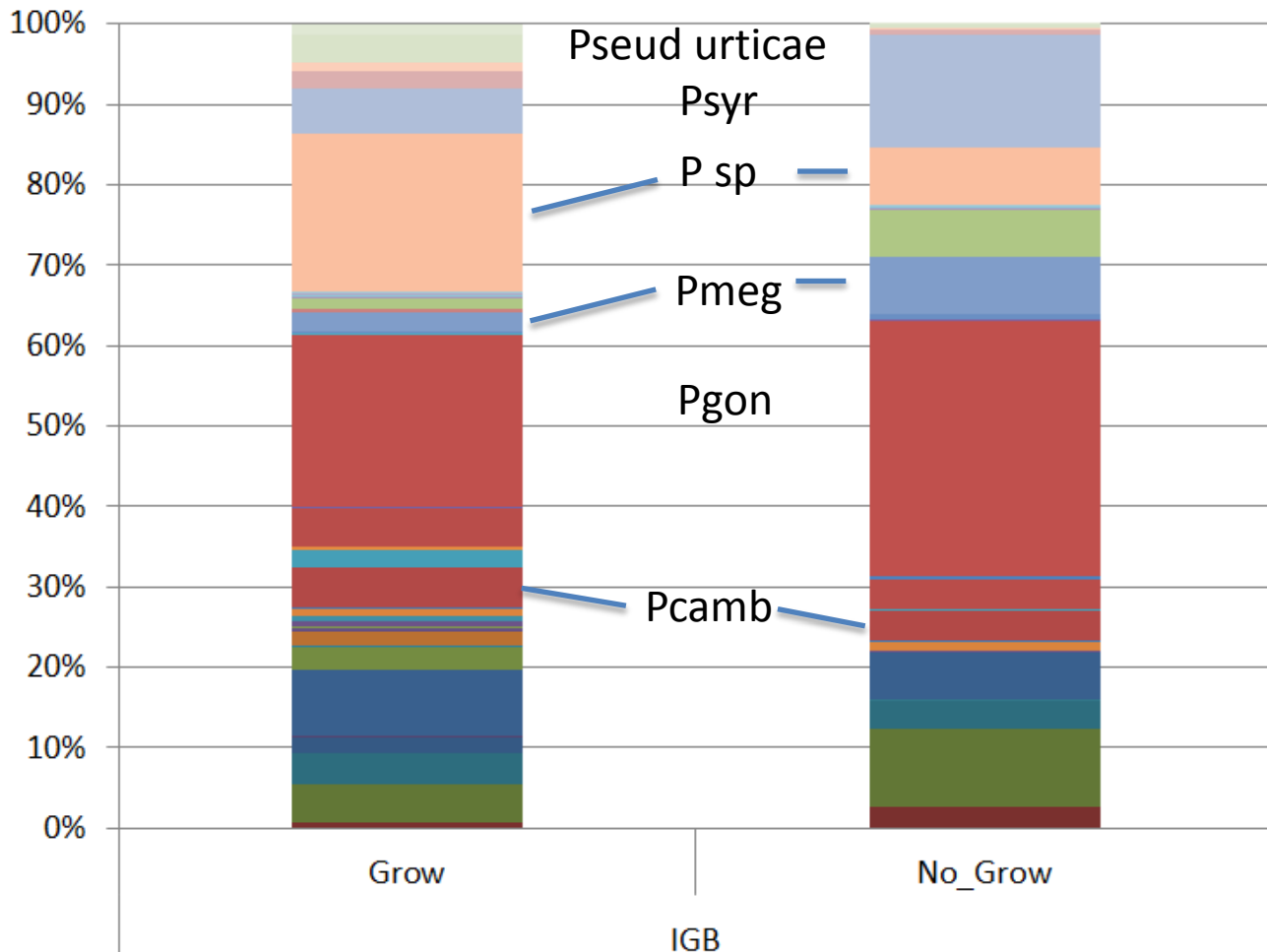
Illumina results (overview)



- Clear site-to-site variation
- Less diversity at GSB Glensaugh (mostly *P. infestans*)
- Most diversity at Invergowrie (more samples and lowland)



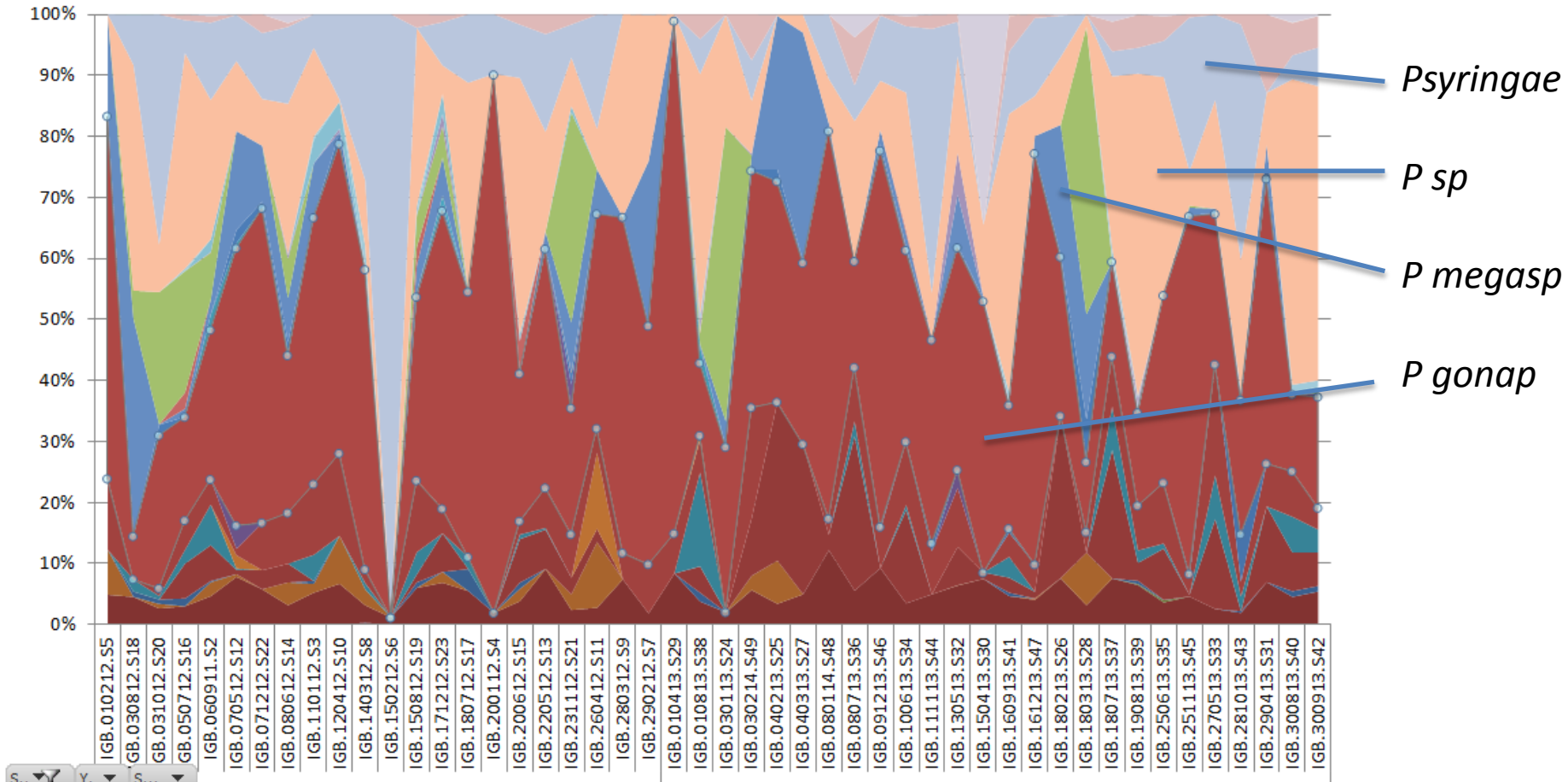
Illumina results (growing season)



- Growing season (April - Sept) – No_Grow (low_grow) Oct - Mar
- *P. gonapodyides* dominates at each. *P. cambivora* in both
- More *P. syringae* over winter
- More *Pseudoperonospora urticae* and *Phy. Sp.* over summer



Illumina results - finer time scale IGB



- *P. gonapodyides* dominates at each time point
- Regular occurrence of some species
- Periodic or sporadic appearance of others



Technology ready?

- The method works well but...
- Laboratory time – still takes weeks to run and analyse the data
- Bioinformatics pipeline needs work to increase speed and confidence
- Costs? Approx. £2000 a run of 96 samples but the costs will fall