

Multisite Phenotyping to Accelerate Willow Breeding & Deployment (AWBD) for Biomass Energy Production

Rust disease, pests (larvae, beetles, aphids), senescence, first year cutback fresh weights, vertebrate (rabbit, hare, deer) and other damage (wind, frost, herbicide, mechanical) have been assessed on a training population of 560 biomass willow genotypes growing at four UK environmentally contrasting sites.

Here, rust, senescence and first year cutback fresh weights data were statistically analysed to provide predicted means for correlations, rankings and use in genomic selection (prediction) modelling.

Correlation graphs show the distribution and differences of across trial sites for these important biomass willow crop traits.

Variability in genotype performance within and between sites and evidence of phenotypic plasticity of some genotypes to adapt in differing environments is observed.

Assessments and analyses continue throughout Year 2 towards our objectives to accelerate willow breeding and deployment.

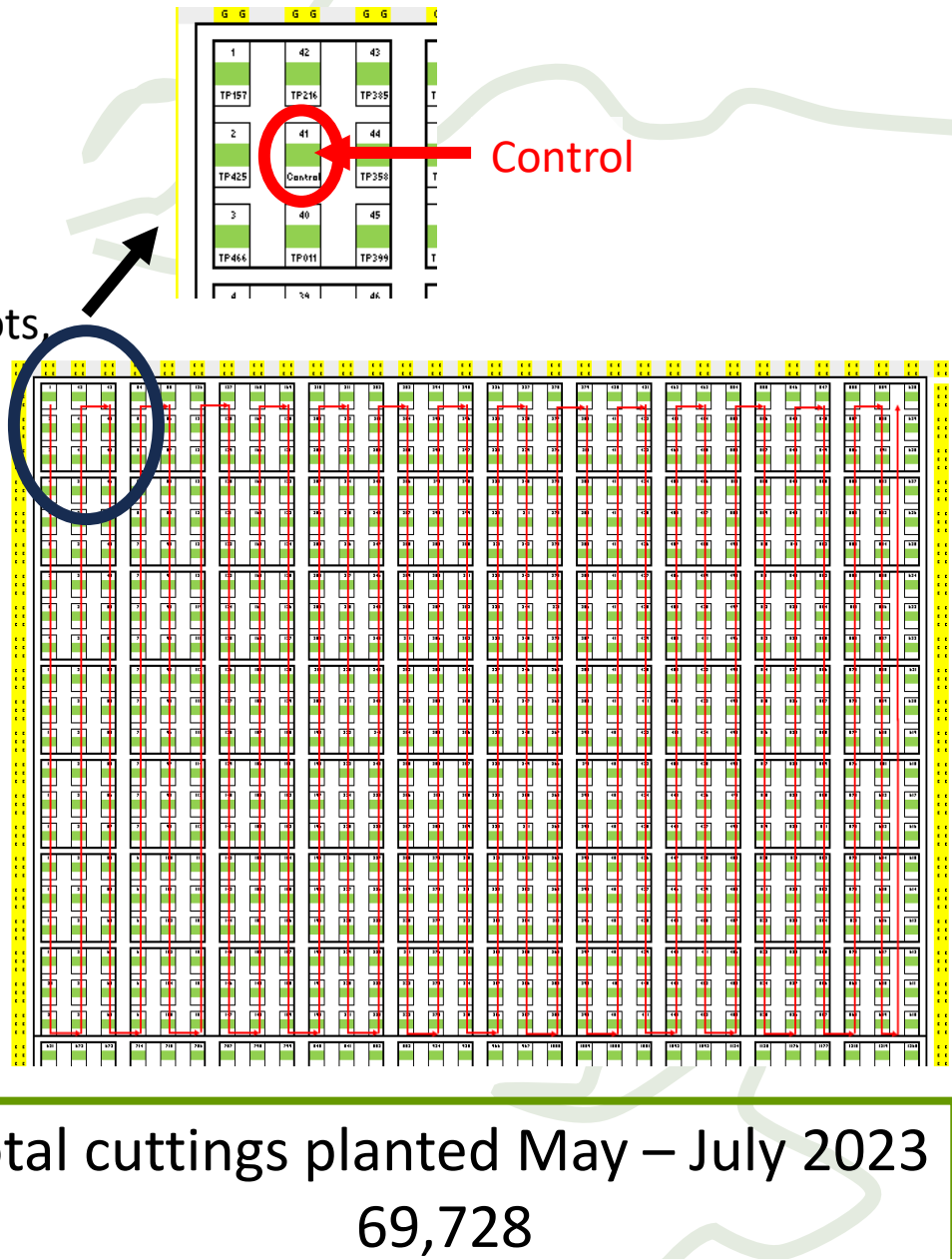
Trial design

Four main Training Population (TP) trial sites:
Aberdeen, Newcastle, Somerset, Woburn

- 80 x 150 m
- 4 replicate blocks, each with 70 sub-blocks of 9 plots, including one control genotype plot per sub-block, enabling adjustment for spatial variation
- 560 genotypes (6 cuttings per plot, per block)
- Guard row surround
- 16,384 cuttings per trial site

One smaller GxE trial site: Hillsborough

- 46 x 65 m
- Similar design
- 141 genotypes (~50% common with TP)
- 4,192 cuttings



Total cuttings planted May – July 2023
69,728

SRUC Craibstone



Phenotyping – traits

Year 1

- Establishment
- Pest damage (insect, rabbit, hare, deer)
- Pathogen damage (rust, *Melampsora spp.*, mildew)
- Aphid presence
- Willow beetles & larvae
- Senescence
- First year cutback & field fresh weights
- Fresh & dry weights for 10 genotypes

Year 2

- Pest damage
- Pathogen damage
- Aphid presence
- Willow beetles & larvae
- Senescence
- Non-destructive yield (height, diameter, shoot no., yield estimate)

Develop precise method for trait(s)
Distribute methods & workbooks
Training online or in person
PHENOTYPING
Receive copy of data in Excel & scan of field scoresheets
Data checking, editing & curation
STATISTICAL ANALYSES

Trait score tables/method

Score	Description
0	No rust found
1	ONE or a few pustules (uredinia)
2	Some/more than a few pustules
3	> score '2'
4	> score '3'
5	Fairly dense pustules
6	Extreme infection, leaf covered in pustules, leaf defoliation often observed
7	Stem rust observed
M	Pustules observed on midrib or petiole

Score	Description
0	Canopy uniformly green – no leaf abscission (there may be a few leaves showing yellow and/or brown patches in the lower layers due to general canopy turnover)
1	Up to 10% of canopy senesced (senescence characterised by leaves turning yellow and/or brown and/or abscising)
2	10-25% of canopy senesced
3	25-50% of canopy senesced
4	50-75% of canopy senesced
5	75-90% of canopy senesced
6	90%+ of canopy senesced
7	Completion of leaf abscission (none/few dead leaves remain hanging)

Plot year cutback & yield (summary)

- Use the measuring guide to cut all shoots from the ground at the centre of the plant to a 1.0 m length, ensuring that the end of each shoot is cut at 50° (i.e. do not cut at a 45° angle)
- Select scales. For larger plants >150 g, use digital hanging balance. For plants <150 g, use the spring balance.
- Check with the digital balance first due to its higher weight capacity (5kg).
- For plants > 150 g, tare the scales EVERY time, with the bucket in place, even if they are showing ZERO.
- For plants <150 g, use 150 g 'as error', with the bucket in place, and tare to 150 g prior to each weighing.
- Record plants as: dead = 'X', missing value/error = '?', weighing less than 2 g = <2, too small to be cut, but alive = <100g.

AND observations such as: vegetative bud burst (BB); 'cankers', 'green leaves' from last season; stem damage (SD), broken/snapped or partially missing shoots (anything that could affect the fresh wt results).

Any cut shoots discarded in error must be marked as a missing value (?). Do not attempt to recover. Try not to damage/stand on any cut plants when moving around the plots.

AFBI-NI Hillsborough

Statistical analyses

- Linear mixed models, fitted using the REML algorithm
- Assessed for differences between genotypes
- Adjusted for spatial variation as captured by the blocking structures
 - Replicates, block-columns, blocks, plots
 - Adjustment aided by systematic inclusion of Control plots
- Analyses for central (main) trees
 - Substitute trees included where central trees dead/unrepresentative – but still some missing observations
 - Mean response per plot across central trees
 - Predicted means provided as input to Genomic Selection modelling

Newcastle University

Cockle Park



Correlation of predicted means between TP sites

Rust - Year 1

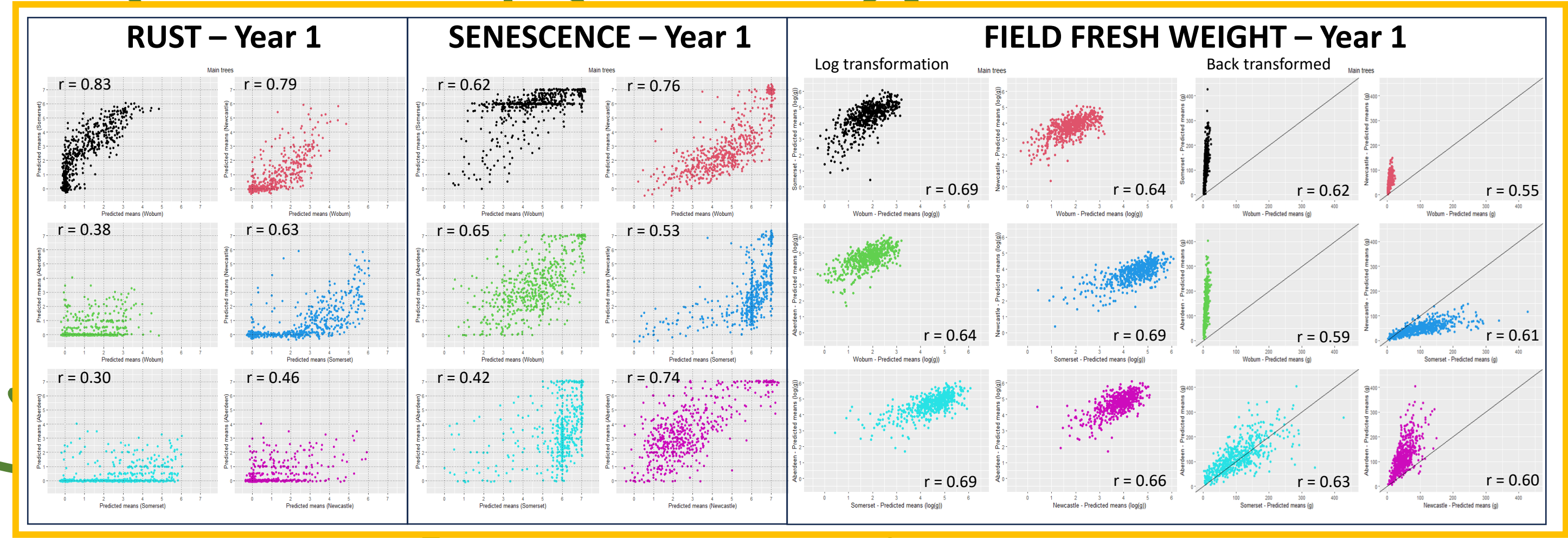
- Good distribution of scores at most sites
- Prevalence of resistant (0) scores at all sites (mostly) from the elite varieties
- Highest scores in Somerset (and wide distribution of scores across genotypes)
- Lowest scores in Aberdeen (assessment was performed earlier here)

Senescence – Year 1

- Good distribution of scores across all sites
- Aberdeen, Newcastle and Somerset assessed on the same two days, with Woburn 1 week later
- Somerset senesced earliest and scores were higher, flooded across the trial when assessed

Fresh weight yield (at 1st year cutback)

- Variation in mean weight yields for all TP & controls as a percentage of the highest yielding site: Aberdeen (100%), Somerset (86%), Newcastle (40%) and Woburn (6%)
- Data shows clearly the weight differences across the trial sites, with some genotypes showing phenotypic plasticity across environments



Rothamsted Research

Woburn Experimental Farm



Somerset Willow Growers

