

Determining management strategies to control ash dieback disease through the study of molecular and environmental interactions

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Figure S1. The research study areas at Rushmore Estate, Southern England (A) and twenty-two experimental plots (B)



Figure S2. Visual assessment images of different crown damage percentage (Raykova, 2019-2021)

Figure legend: Class 0 = trees with no obvious crown damage symptoms; Class 1 = trees with crown damage up to 10 %; Class 2 = crown damage of 10-25%; Class 3 = crown damage of 25-50%; Class 4 = crown damage of 50-75%; Class 5 = crown damage of 75-90%; Class 6 = crown damage of 90-100%; Class 7 = completely dead trees

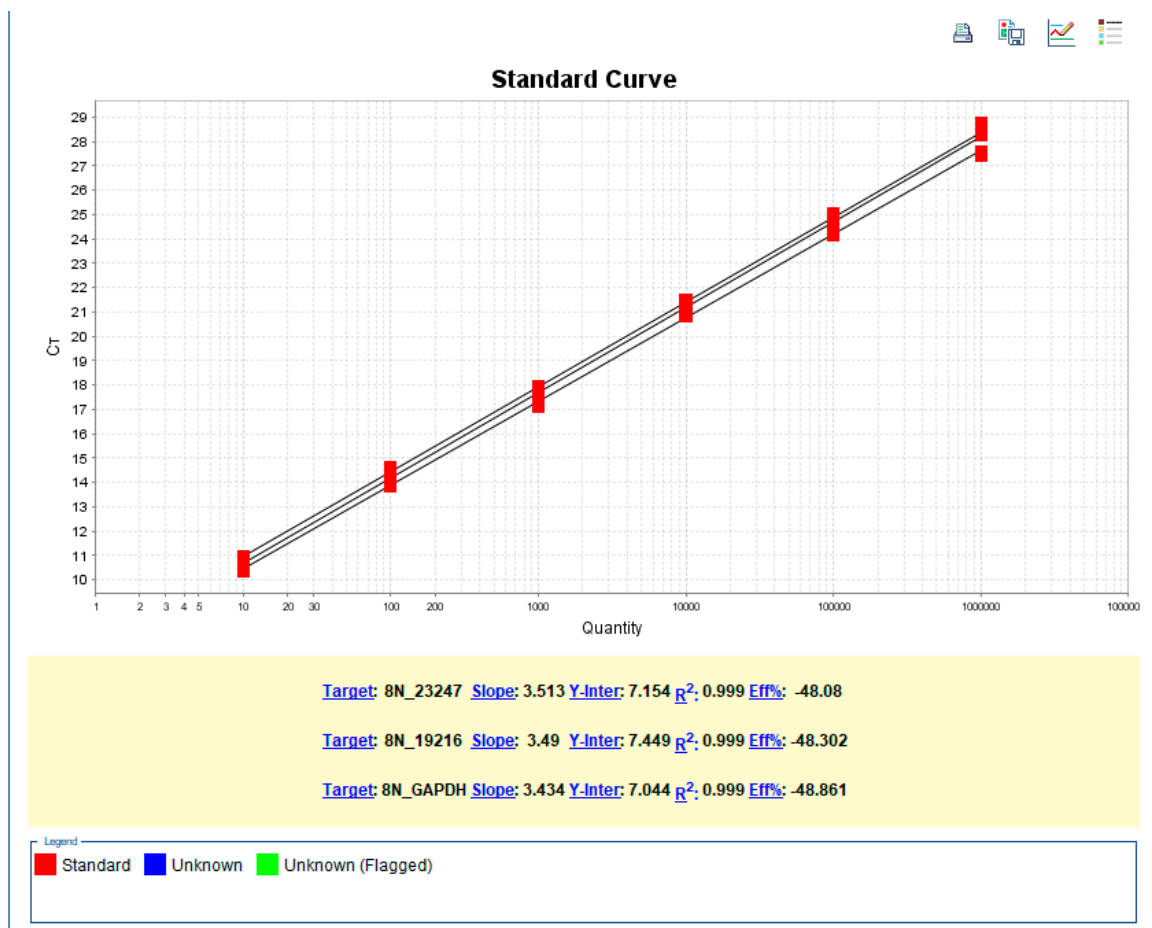


Figure S3. Relative contribution and primer efficiency test for the two target genes Gene_19216 and Gene-23247 and the reference GAPDH.

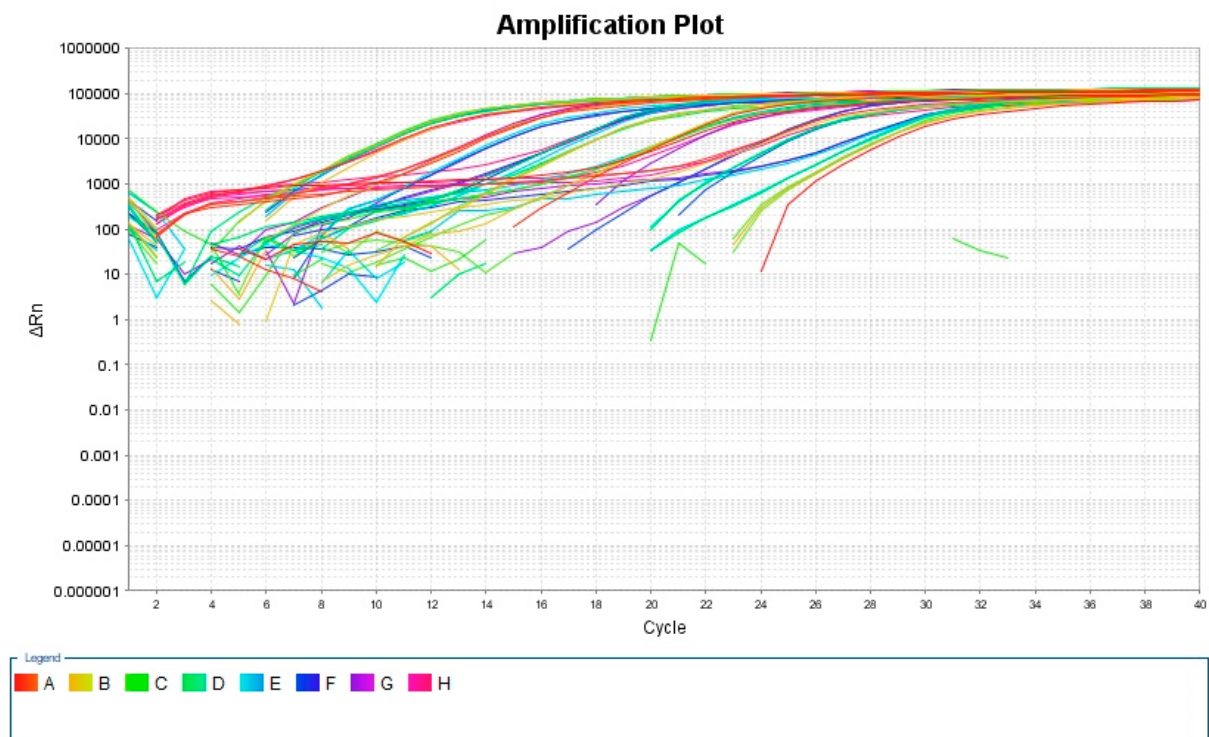


Figure S4. Amplification plot inspection of the primer efficiency test (Figure S3) using Step One Plus software

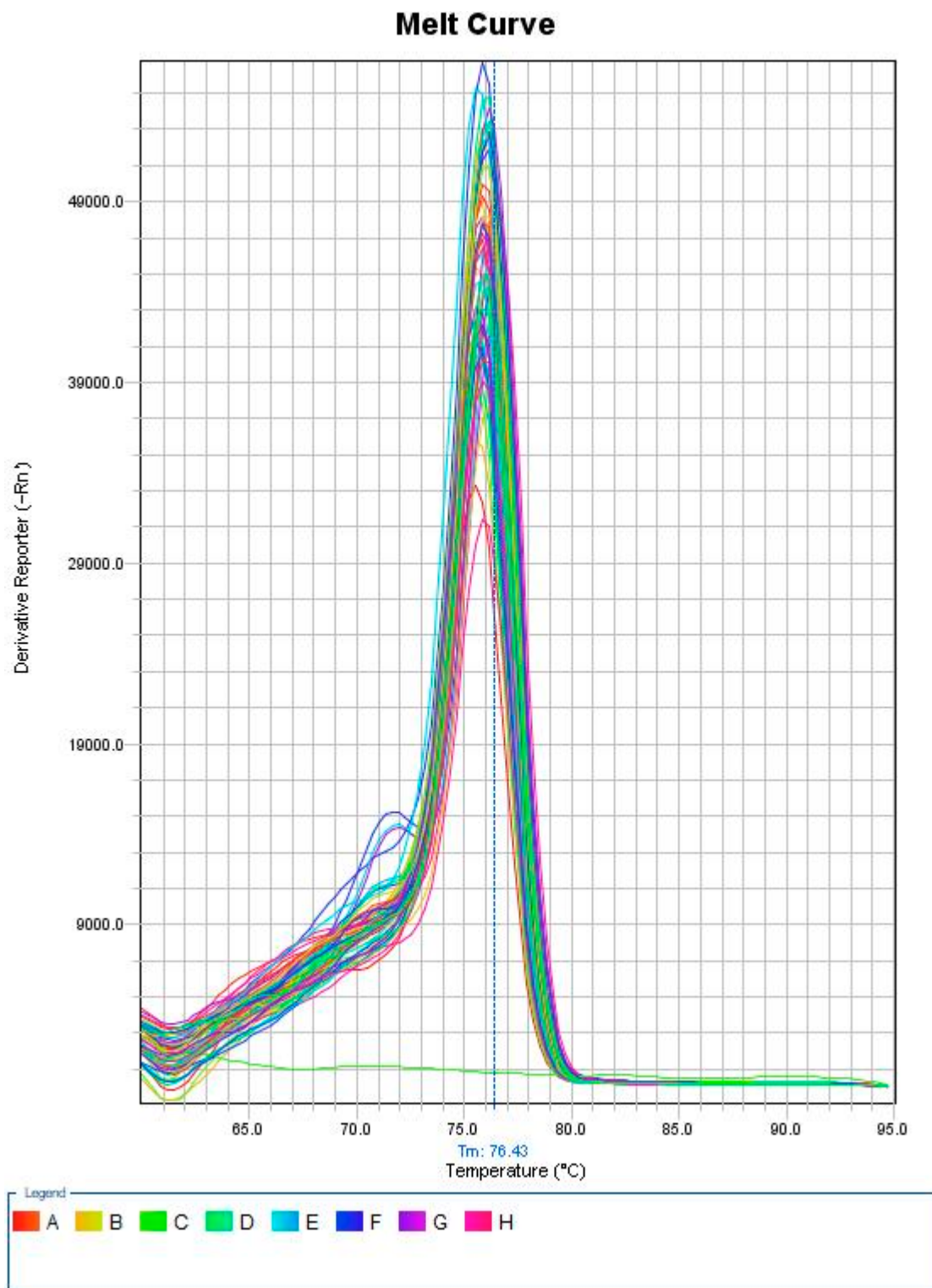


Figure S5. Melt curve inspection for the primer efficiency test (Figure S2) conducted for two target genes: Gene_19216 and Gene-23247 and the reference GAPDH, using Step One Plus software

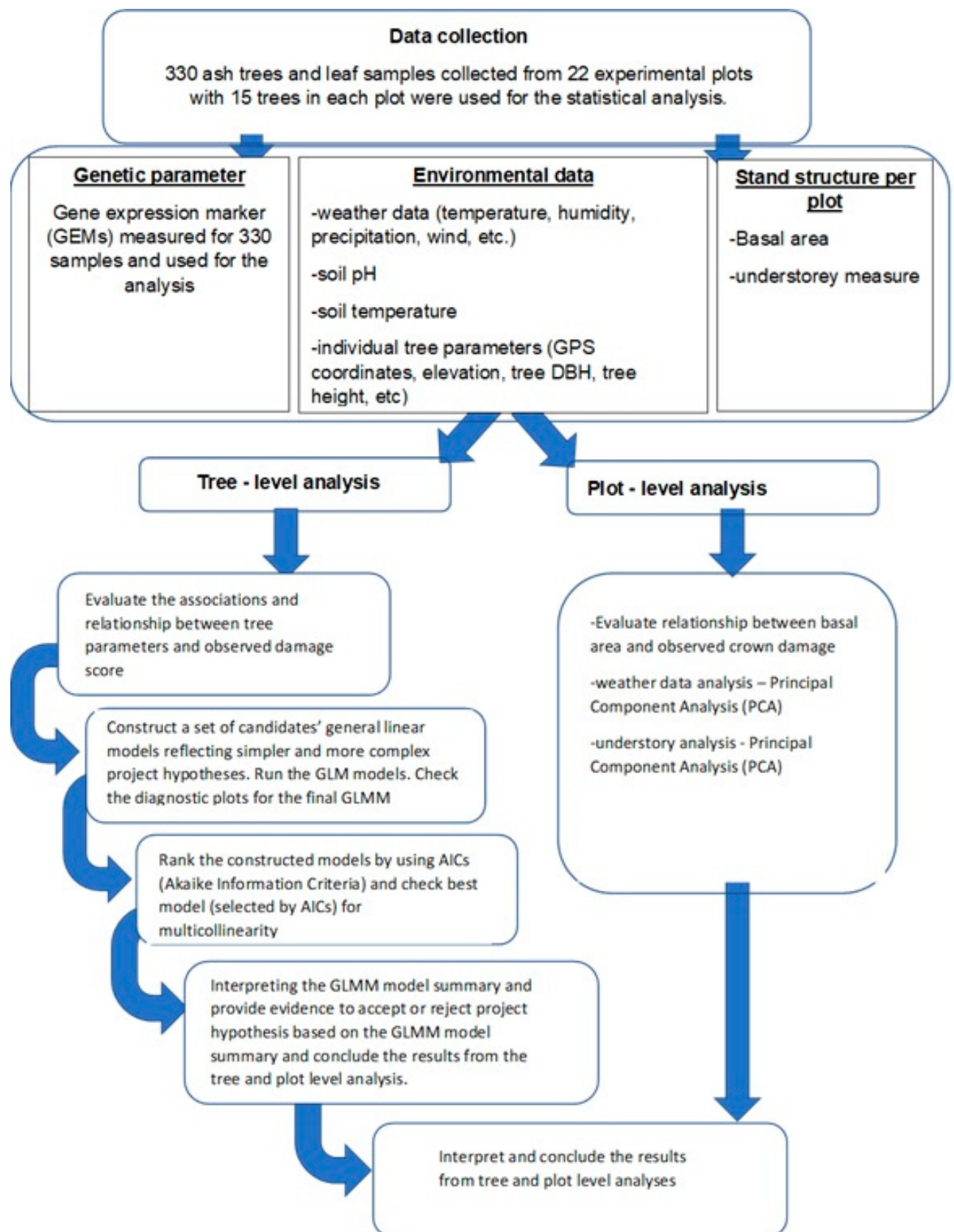


Figure S6. Illustration of the analytical approach for the statistical analysis

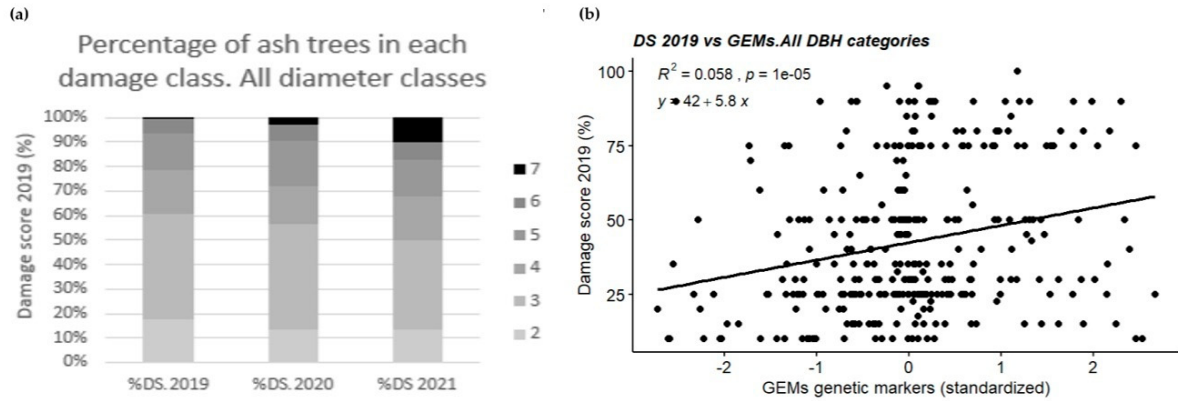


Figure S7. All diameter classes of *Fraxinus excelsior* damage score percentage (a) and the mean of the two (Gene_19216 and Gene_23247) GEMs genetic markers (b); DBH categories: cat.1 (DBH 7-27.99), cat.2 (DBH 28-47.99), cat.3 (48-67.99), and cat.4 (68-86.99)

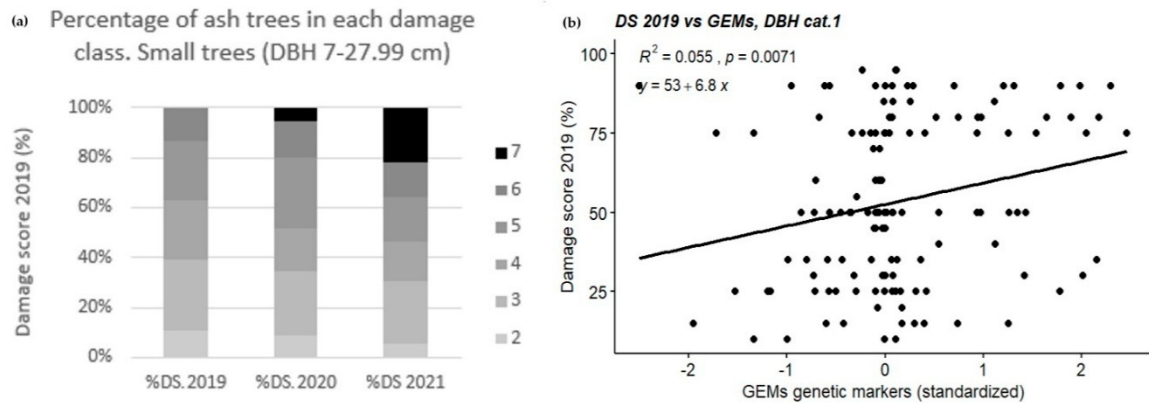


Figure S8. Small size DBH cat.1 (7-27.99 cm) of *Fraxinus excelsior* damage score percentage (a) and the mean of the two (Gene_19216 and Gene_23247) GEMs genetic markers (b)

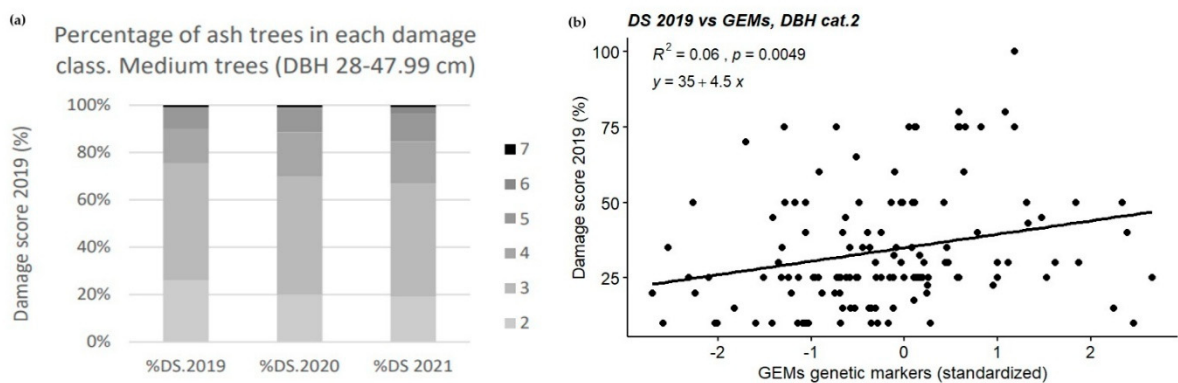


Figure S9. Medium size DBH cat.2 (28-47.99 cm) of *Fraxinus excelsior* damage score percentage (a) and the mean of the two (Gene_19216 and Gene_23247) GEMs genetic markers (b)

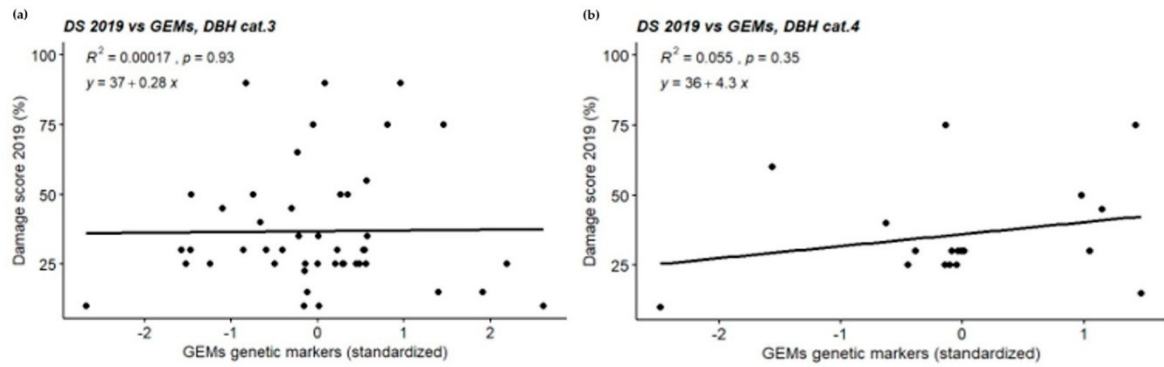


Figure S10. Intermediate size DBH cat.3 (48-67.99 cm) (a) and large size DBH cat.4 (68-86.99 cm) (b) of *Fraxinus excelsior* damage score percentage and the mean of the two (Gene_19216 and Gene_23247) GEMs genetic markers

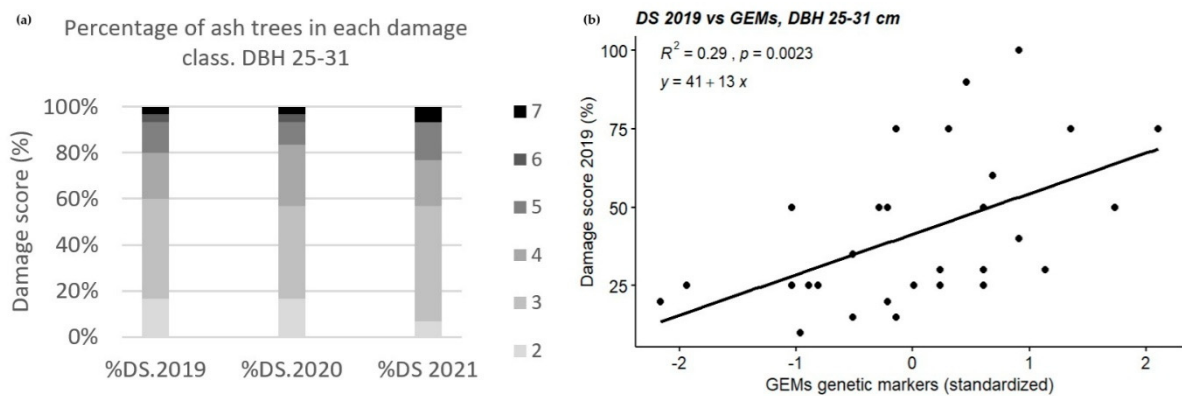


Figure S11. DBH 25-31 cm *Fraxinus excelsior* damage score percentage (a) and the mean of the two (Gene_19216 and Gene_23247) GEMs genetic markers (b)

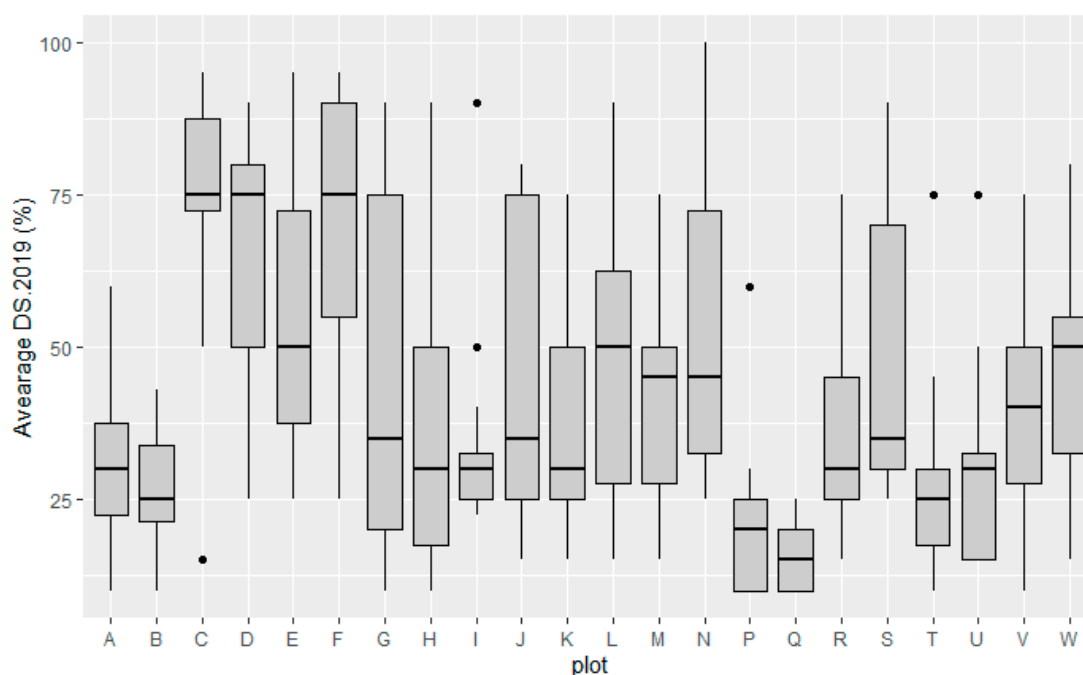


Figure S12. Boxplot comparison of damage score variation among the twenty-two experimental plots at Rushmore Estate. The boxes demonstrate the lower and upper quartiles of the damage scores given for all ash trees assessed 5n the twenty-two plots. The whiskers indicate the variability of the damage scores outside the upper and lower quartiles for each spore plots. The outliers are illustrated as “small circles” representing particular damage scores that differ considerably from the rest of the scores assessed in the plots.

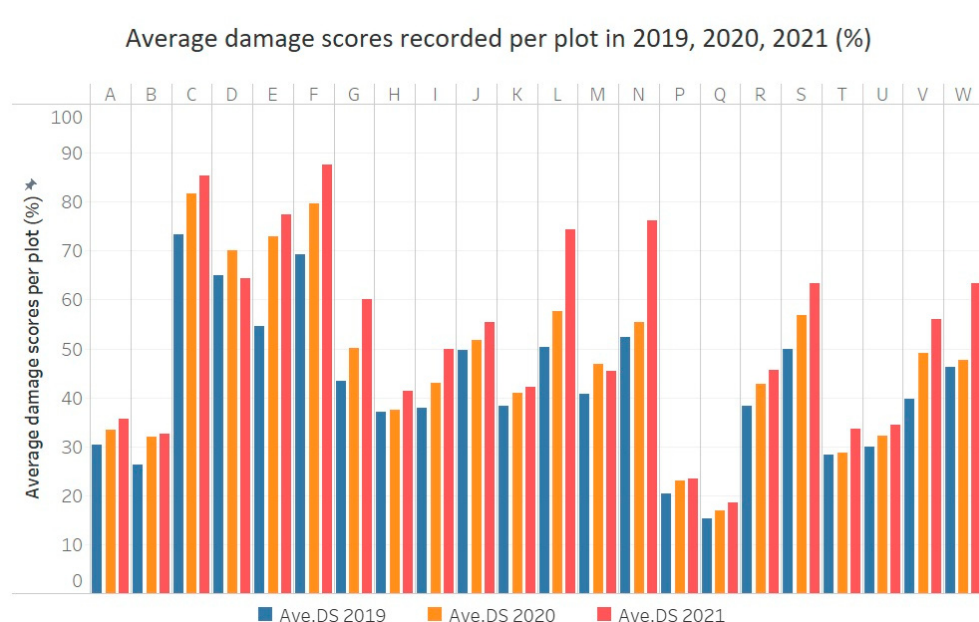


Figure S13. Tree crown average damage score assessments recorded in 2019, 2020, and 2021 (%)

Our analysis for the Progression of the disease observed over three consecutive years at the Estate demonstrated that the crown damage score observed in our 330 experimental ash trees at Rushmore during the period of 2019, 2020, and 2021 (Fig. 4). The correlation coefficient between the three years of crown

damage assessment is significant (2019, 2020, $R=0.95$, $p < 0.001$; 2020,2021, $R= 0.91$, $p < 0.001$; 2019, 2021, $R= 0.87$, $p < 0.001$) suggesting the progression of the disease in those areas.

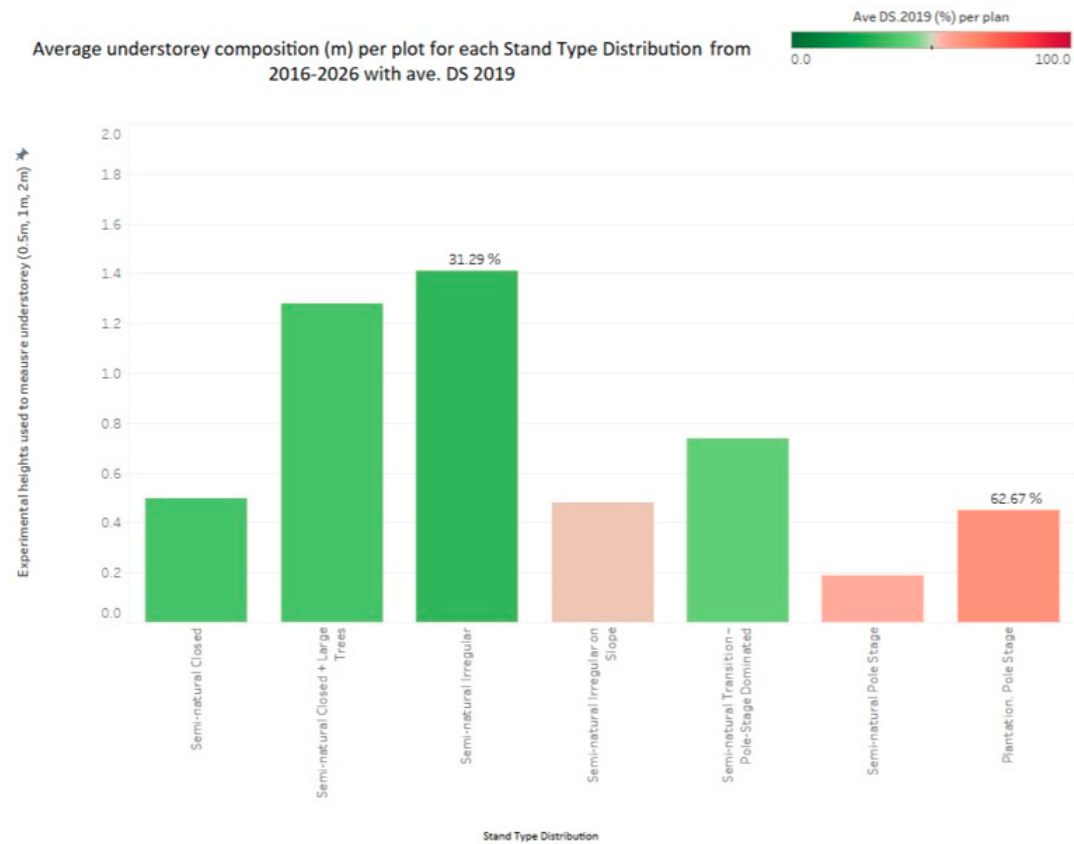


Figure S14. Average understorey composition (m) per plot for each Stand Type Distribution from 2016-2026 with average DS 2019 (colour- coded) showing the variation of understorey vegetation measured at three experimental heights (0.5m, 1.0m, and 2.0m), at each cardinal direction in each experimental plot, covering a minimum of 50% of the plot with measurement points per Stand Type: Semi-natural Closed; Semi-natural Closed and Large Trees; Semi-natural Irregular; Semi-natural Irregular on Slope; Semi-natural Transition- Pole-Stage Dominated; Semi-natural Pole Stage; Plantation, Pole Stage

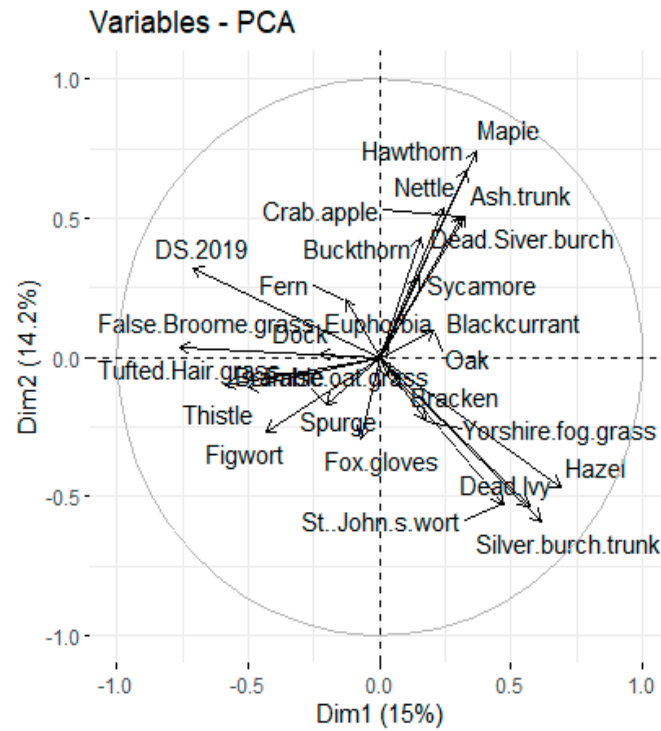


Figure S15. Correlation circle of the understorey species measured in 2019 at the experimental plots at Rushmore Estate

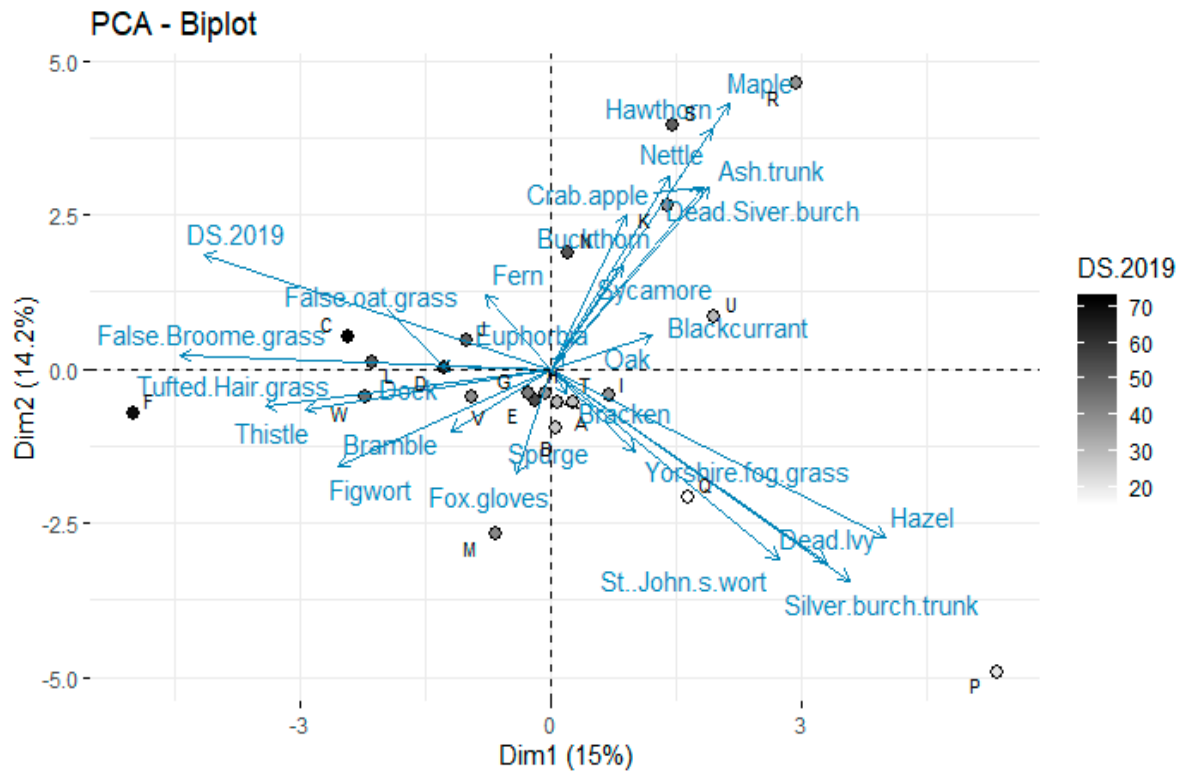


Figure S16. PCA- biplot representing the grouping of the correlated average understorey species and their associated crown damage score measured in 2019

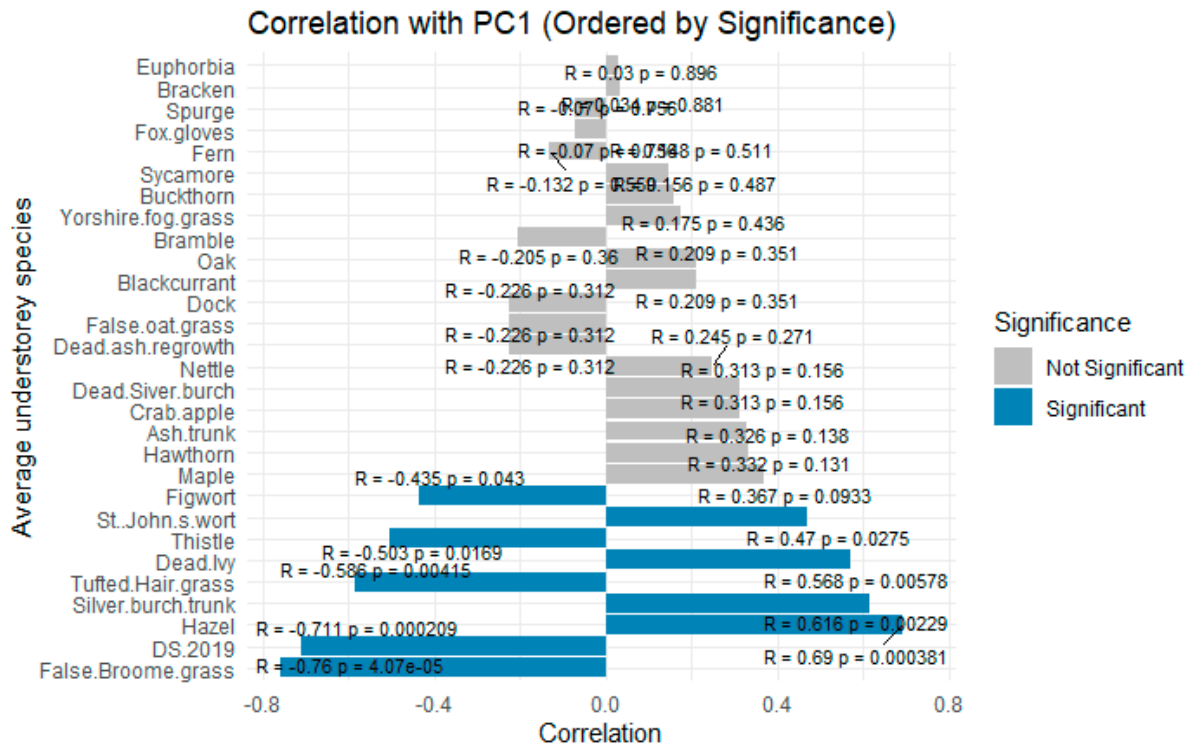


Figure S17. Correlations of the average understorey species and PC1 (ordered and coloured by significance)

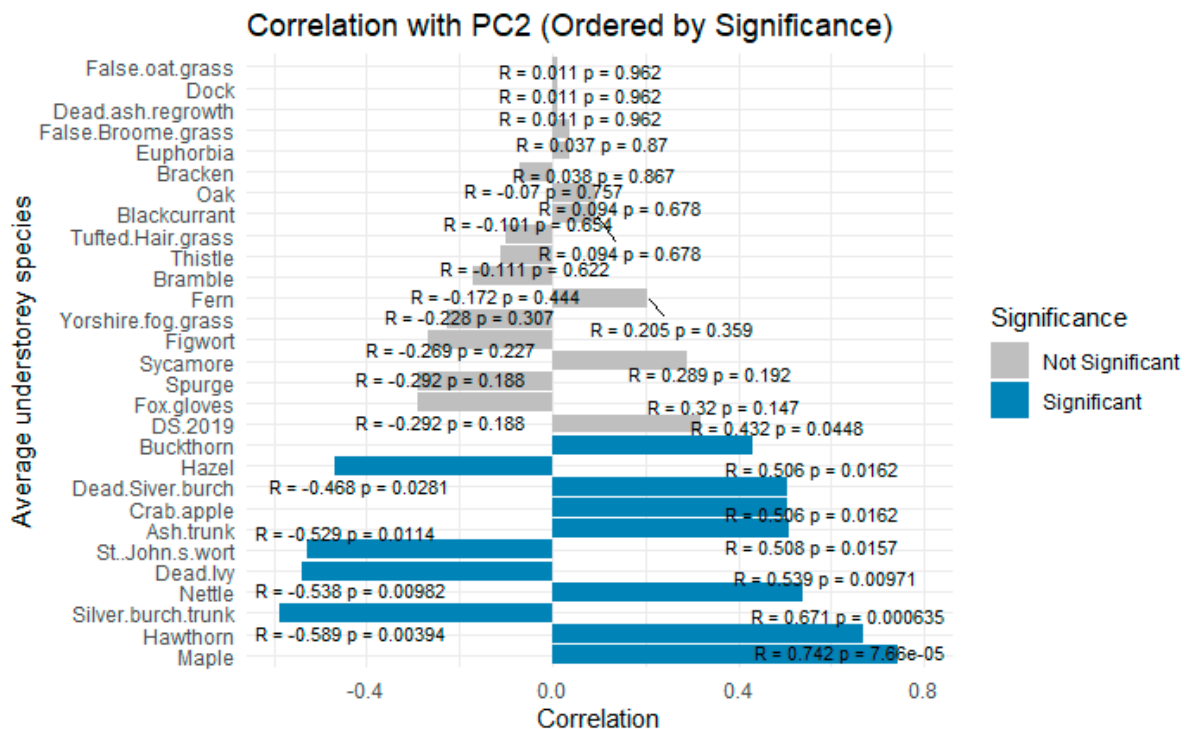


Figure S18. Correlations of the average understorey species and PC2 (ordered and coloured by significance)

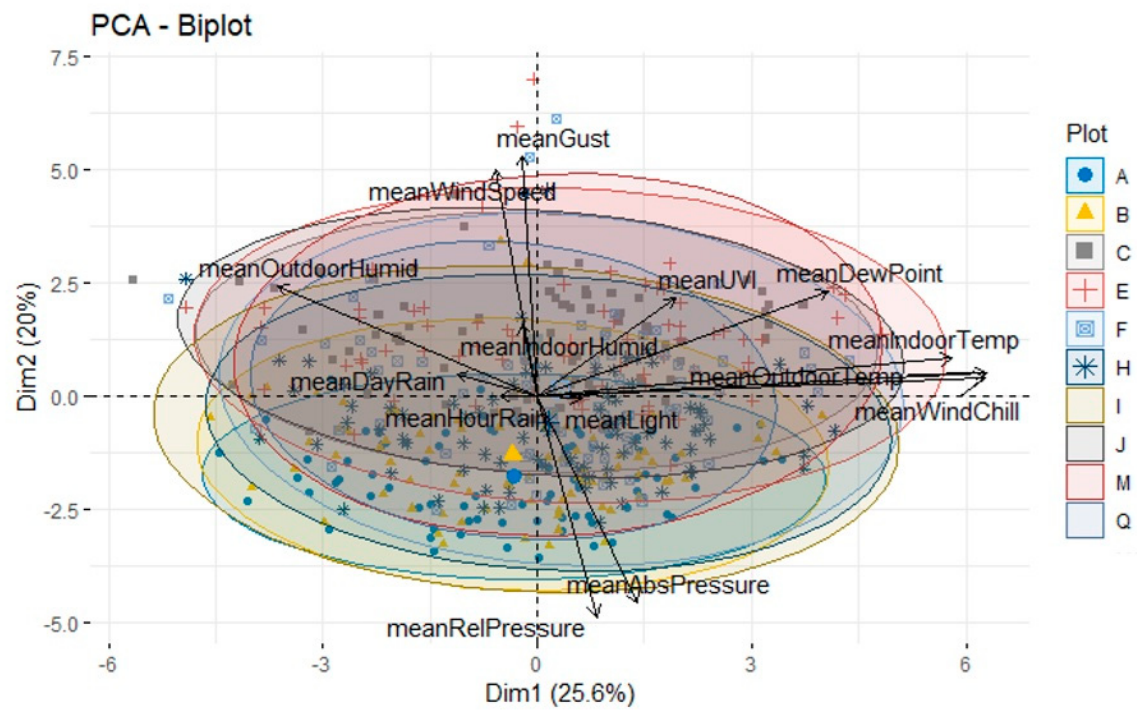


Figure S19. PCA plot showing the weather variables grouped by plot (ellipse)