The physiological, transcriptional and genetic responses of an ozone-sensitive and an ozone tolerant poplar and selected extremes of their F2 progeny

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Ozone-responsive transcriptional changes and genetic control were studied in Populus plants with contrasting ozone sensitivity.

1. Introduction

Tropospheric ozone concentrations have increased by \( \sim 38\% \) since industrial times (Denman et al., 2007) and Fowler et al. (1999) predicted that up to 50\% of forests will be exposed to 60 nL L\(^{-1}\) ozone by 2100, a concentration known to damage plants (Miller et al., 1963). For agricultural and forest ecosystems, tropospheric ozone is considered the most widespread and damaging pollutant (Lindroth, 2010; Ludwikow et al., 2004), and a down-regulation of photosynthetic components (Conklin and Last, 1995; Glick et al., 2003) and stress related genes and compounds (Conklin and Last, 1995; Glick et al., 2003) have long been a commercially important genus, with diverse uses such as timber, pulp and paper, carbon sequestration, a biofuel feedstock and bioremediation. More recently it has joined the league of model organisms (Brunner et al., 2004; Taylor, 2002; Wullschleger et al., 2002) and is the first tree to have a fully sequenced genome (Tuskan et al., 2006). Microarrays constructed from Populus EST collections provide the opportunity to probe the transcriptional response to ozone in Populus, a perennial deciduous tree. In a recent meta-analysis Populus also exhibited similar responses in terms of biomass loss to a number of major keystone forest species (Wittig et al., 2009).
where $C$ is the general mean, $T_j$ is the genotype by treatment interaction and $e_{ijk}$ is the error.
3. Results and discussion

3.1. P. deltoides and P. trichocarpa grandparents show contrasting responses to acute and chronic ozone exposure

Acute ozone exposure induced marked differences in visible symptoms between the two grandparental species (Fig. 1a). P. trichocarpa leaves began to develop lesions within nine hours which spread and blackened with further exposure. No such symptoms were observed in P. deltoides at any point time. Chronic fumigation significantly increased the percentage of abscised leaves in both species, but to a greater extent in P. deltoides on day 30. Ozone significantly reduced chlorophyll content in P. deltoides, with a slight non-significant increase shown for P. trichocarpa.

![Fig. 1. The progression of ozone damage for P. deltoides and P. trichocarpa exposed to 200 nL L\(^{-1}\) ozone in growth chambers for 5 days, showing necrotic lesion formation in P. trichocarpa, with a lack of visible symptoms in P. deltoides. b][]{The response of three ozone tolerant and three sensitive F\(_2\) genotypes of family 331 to a 9 h exposure of 200 nL L\(^{-1}\) ozone in growth chambers, showing necrotic lesions in the sensitive clones.}

For growth traits, there was generally a negative effect of ozone, with height, diameter, leaf number, and leaf expansion rate all showing a decrease in both grandparental species (Table 1). P. trichocarpa exhibited considerably more visible damage than P. deltoides, with 51% of leaves showing characteristic ozone damage, compared to 0% for P. deltoides. Lesion morphology was similar to that seen in the acute treatment suggesting that the same physiological response is involved in the formation of lesions in both chronic and acute treatments. These results suggest that the two grandparental species show significantly different responses to ozone exposure. The rapidly spreading lesion morphology of P. trichocarpa that resulted in complete death of leaves was striking, especially in contrast to the P. deltoides grandparent.

3.2. An F\(_2\) mapping pedigree exposed to a chronic ozone treatment

For all traits, there was a highly significant effect of genotype (\(p < 0.0001; \) Table 2). No significant ozone effect on height was found but in contrast for basal stem diameter a small (\(-3.2\%\)) but significant negative effect (\(F_{1,122} = 11.24, \ p < 0.001\)) of ozone was found similar to that reported in a meta-analysis of forest tree ozone responses (Wittig et al., 2009), indicating that ozone exposure is likely to impact biomass yield. This will require further confirmatory analysis from longer-term studies, but suggests that Populus biomass yield is likely to be detrimentally affected by future increased ozone concentrations, which are consistent with previous reports (Bohler et al., 2007; Bortier et al., 2000; Karnosky et al., 1996; Wittig et al., 2009; Woo and Hinckley, 2005). Such decreases in productivity are likely to be related to decreased photosynthetic activity (Bortier et al., 2000; Coleman et al., 1995; Degl’Innocenti et al., 2007; Lorenzini et al., 1999) and reduced leaf area (Wittig et al., 2009).

Ozone had a positive influence on the total number of leaves initiated both early (\(F_{1,123} = 4.62, \ p < 0.05\)) and late (\(F_{1,125} = 5.45, \ p < 0.05\)) in the season. The area of the first unfurled leaf was significantly greater in ozone (\(F_{1,93} = 6.82, \ p < 0.01\)), whilst leaf area expansion rate was significantly reduced (\(F_{1,93} = 7.82, \ p < 0.01\)). No significant effect on final leaf area was found. There was a highly significant increase in leaf abscission. Chlorophyll content and late percentage abscission (70d) showed significant genotype\(\times\)treatment interaction, suggesting that differential response mechanisms exist within the F\(_2\) genotypes (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control</th>
<th>Ozone</th>
<th>% Effect</th>
<th>Trt</th>
<th>Control</th>
<th>Ozone</th>
<th>% Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (cm)</td>
<td>55.30</td>
<td>53.00</td>
<td>−4.34</td>
<td>ns</td>
<td>79.30</td>
<td>72.30</td>
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<tr>
<td>Diameter (mm)</td>
<td>6.03</td>
<td>5.43</td>
<td>−10.00</td>
<td>ns</td>
<td>6.47</td>
<td>5.17</td>
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<tr>
<td>Leaf number (30d)</td>
<td>15.70</td>
<td>14.70</td>
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<td>ns</td>
<td>19.70</td>
<td>15.30</td>
<td>−28.76</td>
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<tr>
<td>Leaf number (70d)</td>
<td>21.30</td>
<td>18.30</td>
<td>−16.30</td>
<td>*</td>
<td>26.70</td>
<td>26.00</td>
<td>−2.69</td>
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<tr>
<td>Chlorophyll content</td>
<td>15.10</td>
<td>9.53</td>
<td>−58.45</td>
<td>**</td>
<td>20.70</td>
<td>21.90</td>
<td>5.48</td>
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<tr>
<td>% Absceded (30d)</td>
<td>7.10</td>
<td>17.60</td>
<td>99.66</td>
<td>***</td>
<td>8.80</td>
<td>10.30</td>
<td>14.56</td>
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<tr>
<td>% Absceded (70d)</td>
<td>18.50</td>
<td>40.00</td>
<td>53.75</td>
<td>***</td>
<td>22.80</td>
<td>53.90</td>
<td>57.70</td>
</tr>
<tr>
<td>Area of first unfurled leaf (cm)</td>
<td>7.40</td>
<td>6.64</td>
<td>−11.38</td>
<td>*</td>
<td>9.94</td>
<td>12.62</td>
<td>21.20</td>
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<td>Leaf expansion rate (% increase)</td>
<td>74.60</td>
<td>68.20</td>
<td>−9.38</td>
<td>ns</td>
<td>87.20</td>
<td>54.40</td>
<td>−60.29</td>
</tr>
</tbody>
</table>

We used an FDR-adjusted \(p\) value of 0.05 as our threshold for declaring an EST as differentially expressed.

The list of significantly differentially expressed ESTs was then median-aggregated on a gene model basis to yield a final list of differentially expressed genes. ESTs were assigned to ‘jamboree’ gene models from v1.1 of the P. trichocarpa ‘Ni- qually-1’ genome sequence (http://genome.jgi-psf.org/Poptr1_1/Poptr1_1.home.html) based on filtered BLAT (Kent, 2002) alignments. ESTs with low BLAT scores were removed to avoid incorrect assignment to gene models. Arabidopsis thaliana orthologs were then identified by BLASTp searches of the predicted Populus gene model protein sequences against the protein sequences in TAIR release 7 (The Arabidopsis Information Network, www.Arabidopsis.org). Results with low BLASTP scores were left blank to indicate that no ortholog could be identified.

To identify genes with differential expression between the tolerant and sensitive \(F_2\) genotype groups, Partial Least Squares Discriminant Analysis (PLS-DA) was performed in the software package SIMCA P (Umetrics Ltd, Windsor, UK). Normalised data were imported and scaled by mean centring. A Variable Importance (VIP) score was generated for each EST on its ability to explain the separation between groups. The top 50 ESTs were taken to be differentially expressed, and all exceeded a VIP score of 2. This list was then aggregated to representative gene models for the purpose of examining QTL co-location.

Functional over-representation analysis was performed using the BINGO plugin (Maere et al., 2005) for the network visualisation software Cytoscape (version 2.5.1, Shannon et al., 2003). We used the hypergeometric test and set a Benjamini and Hochberg FDR-adjusted significance level of 0.05 for declaring a GO (Gene Ontology, www.geneontology.org) category as significantly over-represented. As there is not yet a mature GO release for Populus, we used the best BLASTp hit results of predicted Populus gene models to A. thaliana to infer GO using the TAIR 7 release of the A. thaliana genome. Tests were performed using the Arabidopsis thaliana GO-plant slim ontology.

Expression data from the microarray analysis was validated for selected targets using RT-qPCR, this is described in the Supplementary materials and methods.
3.3. QTL mapping in the F2 population

In total, 58 QTL were identified for 11 traits (Table 3) and were found on all linkage groups except VII and XVI. Individual QTL explained between 1.5 and 16.3% of phenotypic variance, with the lowest being for basal stem diameter in ozone on LG Vb, and the highest for visible damage in ozone (late season) on linkage group X. These estimates of explained phenotypic variance represent over-estimates due to the Beavis effect (Utzi et al., 2000; Xu, 2003).

The average confidence interval span was 30 cM, with the smallest being 4 cM for chlorophyll content in response to ozone on LG XVII. Where traits were measured on two occasions, this is indicated by 30 d (30 days of exposure) or 70 d (70 days of exposure). Treatment abbreviations in bold indicate that these represent genomic regions that are specific.

3.4. Sensitive and tolerant F2 genotypes exposed to an acute ozone treatment

The three most sensitive and three most tolerant genotypes were selected on the basis of visible damage and necrotic lesion development after 30 and 70 days of the chronic ozone exposure. No significant differences were seen between sensitivity groups for any of the traits except visible damage at 30 and 70 days and those relating to leaf size (Table 4). It is evident from the trait data for the sensitive and tolerant genotypes (Tables 1–3) that the selection of F2 genotypes at the population extremes for the trait of interest (viscous leaf damage) reduced the number of traits showing treatment responses to ozone exposure. This is in contrast to P. deltoides and P. trichocarpa, which exhibited differences for numerous traits besides visible damage. Removing the influence of other traits is

### Table 2
Summary of traits measured in the F2 population in response to a chronic 100 nL L⁻¹ ozone treatment. Where traits were measured on more than one occasion, this is indicated by 30d and 70d (30 or 70 days of exposure). The data represent means for each trait recorded in ambient conditions and elevated ozone, and the percentage effect of ozone upon the trait. A general linear model was used to analyse all data except % abscised (30d) where a Kruskal–Wallis test was performed due to a non-normal distribution. Significance levels are indicated by * p < 0.05, ** p < 0.01, *** p < 0.001.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Control Ozone % effect Trt Geno Geno*Trt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (mm)</td>
<td>7.09 6.57 3.20 *** *** ns</td>
</tr>
<tr>
<td>Leaf number (70d)</td>
<td>26.10 27.15 3.84 *** *** ns</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>21.06 21.22 0.78 ns *** *</td>
</tr>
<tr>
<td>% abscised (30d)</td>
<td>2.45 12.77 80.78 *** *** *</td>
</tr>
<tr>
<td>% abscised (70d)</td>
<td>14.59 33.24 56.11 *** *** *</td>
</tr>
<tr>
<td>Area of first unfurled leaf (cm)</td>
<td>10.56 13.61 10.60 *** *** ns</td>
</tr>
<tr>
<td>Leaf expansion rate (% increase)</td>
<td>99.80 90.70 −10.03 *** * ns</td>
</tr>
</tbody>
</table>

### Table 3
QTL mapped for physiological traits using composite interval mapping on 164 genotypes of Family 331 in 100 nL L⁻¹ ozone (T), ambient air (C), and percentage effect response to ozone (R), showing linkage group (LG) and centiMorgan position (position (cm)), the confidence interval of the QTL (CI), the statistical significance of the QTL (p value) and the percentage phenotypic variance explained by the QTL (%Vp).

<table>
<thead>
<tr>
<th>Trait</th>
<th>C/T/R LG</th>
<th>Position (cm)</th>
<th>CI</th>
<th>%Vp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of first unfurled leaf</td>
<td>C</td>
<td>VIII</td>
<td>0</td>
<td>0−4</td>
</tr>
<tr>
<td>Basal stem diameter</td>
<td>C</td>
<td>X</td>
<td>0.2</td>
<td>0−13.2</td>
</tr>
<tr>
<td>Chlorophyll content</td>
<td>C</td>
<td>I</td>
<td>82</td>
<td>64−96</td>
</tr>
<tr>
<td>Height</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
desirable when studying a single trait, in this case visible damage (Borevitz et al., 2003).

We were interested to see whether these genotypes would also separate as extremes when exposed to acute ozone. The sensitive genotypes showed more severe necrotic damage than *P. trichocarpa*. The tolerant clones remained symptom free (Fig. 1b) suggesting that, at the level of visible damage, these genotypes represent sensitivity extremes in response to both chronic and acute ozone damage.

### 3.5. Stomatal conductance responses to ozone

For the grandparents in the chronic exposure experiment, ozone treatment had no significant effect on stomatal conductance of young leaves. For semi-mature leaves, a significant treatment effect was found (*p* < 0.05). Both grand-parental species showed decreased stomatal conductance in response to acute ozone treatment (*p* < 0.03). *P. deltoides* showed a more rapid stomatal closure response with a significant decrease after three hours compared with nine hours for *P. trichocarpa*. However, after 28 h of exposure, there appeared to be a compensation response with conductance returning to control levels in both species. For the F2 extreme genotypes under chronic ozone exposure, a significant treatment effect was found for semi-mature leaves, with ozone reducing conductance (*p* < 0.005). Conductance did not depend on sensitivity group, and there was no sensitivity group*treatment interaction (data not shown). For young leaves, no terms in the test were significant.

### 3.6. Microarray analysis of the acute ozone response

Both the grandparental species and the F2 extreme sensitivity genotypes showed the most dramatic separation for visible damage in response to acute ozone exposure, their transcriptional response under this stress regime was examined.

Analysis of control-treatment response in the grandparental species identified 1409 genes as differentially expressed in response to ozone. 770 of the genes were up-regulated and 628 were down-regulated, with log2 fold-change in expression ranging from 3.1 to −3.5 (Table S1). We examined the Plant GO-Slim Gene Ontology (GO) categories of the up- and down-regulated ozone responsive genes to provide a broad overview of gene functions active in response to acute ozone exposure (Fig. S1a and b). Photosynthesis and plastid (chloroplast) function were the most represented down-regulated categories, as well as a decrease in metabolism categories such as protein biosynthesis and carboxylate metabolism. The most represented up-regulated categories were those involved in biotic and abiotic stress responses, and secondary metabolism. Table S5 provides details of the 50 most significantly differentially expressed genes in response to ozone.

Analysis of control-treatment response in common to both F2 sensitivity groups of genotypes identified 813 genes as being differentially expressed in response to ozone (Table S2). Of these 477 were up- and 336 down-regulated, the log2 fold-change in expression ranged from 4.5 to −2.3. We also performed an analysis of the response to ozone in each group of genotypes separately. This revealed that when considering only the tolerant genotypes, no genes were significant, whereas 1199 genes were significantly differentially expressed in the sensitive group. The range of M (log2 ratio) values was considerably smaller for the tolerant genotypes than for the sensitive ones (the sensitive genotypes had M values ranging from 4.9 to −3.4). Fig. S1c and d shows the overlap for up- and down-regulated genes respectively between the analysis of the two grandparents, the combined analysis of the two groups of F2 genotypes, and the sensitive group alone. It is clear that although there was significant overlap between the different transcriptional responses, there was also clear separation between the grandparental responses and the response of the F2 sensitive and tolerant genotypes. Gene lists of the overlap between categories can also be found in Table S3. Visual examination of the tolerant genotype dataset revealed that the lack of significant genes was the result of a more inconsistent response across the three genotypes within this group (data not shown). This suggests that although these genotypes have similar leaf-level development of visible damage in response to ozone exposure, the development of those symptoms may result from different transcriptional mechanisms. A selection of four genes showed congruence between RT-qPCR and both microarray platforms for *P. trichocarpa* while this agreement was less for *P. deltoides* with expression patterns of two genes exhibiting variability between microarray platforms (Fig. S2)

### 3.7. Genes with differential expression between ozone sensitivity genotypes

Although it is likely that different transcriptional responses are involved in response to acute and chronic ozone exposure, we were interested to see whether any of the genes showing differential response patterns between the extreme genotypes co-located to mapped QTL. Table S4 details the 36 genes that showed the most significant divergence in response to ozone between the sensitive and tolerant genotypes, as identified using Partial Least-Squares Discriminant analysis. This is a supervised multivariate analysis method similar to PCA but where information on grouping is specifically provided. This method therefore maximises the separation between sample groups. Eleven genes were found to co-locate to ozone treatment or response QTLs, including three on LG X that co-locate to an identified hotspot of QTLs associated with height, leaf number and biomass (Table 3). The co-location of the diameter QTL specific to ozone treatment (LG X) within the same location as *Populus Biomass Loci 3 (PBL-3)* from Rae et al. (2009) suggests that ozone could have an interaction effect on the role of this QTL in biomass accumulation. Fig. 2 shows a subset of linkage groups that contained genes that were differentially expressed in response to ozone between the sensitive and tolerant genotypes and that co-located to mapped QTL. Details of the genes presented in Fig. 2 can be found in Table 6.
Three genes co-located to a QTL for total damage (end-season damage) on LG I. These genes code for UDP-xyl synthase 5 (UXS), a tonoplast-intrinsic protein, and arabinogalactan protein 15. UDP-xyl synthase is involved in the production of many cell wall products as well as the upstream inhibition of enzymes (Schultz et al., 2000). The tonoplast-intrinsic protein is a putative aquaporin, but of unknown function. Arabinogalactan proteins are extracellular proteoglycans that appear to have an as yet unexplained role in influencing cell proliferation (Schultz et al., 2000). Arabinogalactanins have also been shown to be highly expressed in tension wood in poplar (Lafargue et al., 2004). Their role there is suggested to be structural and it is highly probable that ozone is causing structural changes to cell wall components either directly or through the formation and action of free radicals (Fry et al., 2001) and thus inducing stress-associated cell wall re-modelling.

A gene encoding SAM Synthetase (SAMs), a gene involved in the production of ethylene, co-located to QTL for both late and early season visible damage on LG II and showed significant divergence in response between the extreme genotypes and the two grandparental species. S-adenosyl methionine (SAM) is the precursor molecule to both polyamines and ethylene (Langebartels et al., 1991; Overmyer et al., 2005), both of which are thought to be involved in the response to ozone (Langebartels et al., 1991; Overmyer et al., 2005). It is of interest, therefore, that SAM Synthetase (SAMs) co-located to QTL for both late and early season visible damage on LG II. Pandey et al. (2000) put forward a hypothesis for the interacting role of poliozone and ethylene in the control of senescence, with ethylene acting as a positive regulator and polyamines as a negative regulator. The authors suggested a model in which polyamine and ethylene biosynthetic pathways...
compete for a limited pool of SAM, with the interaction between the two determining the outcome. They also postulated that the product of one pathway could act to inhibit the opposing pathway. This presents the intriguing possibility that a similar mechanism could exist in the response to ozone, with the interaction between polyamine and ethylene biosynthesis serving to determine the extent of visible damage.

Although lying outside the mapped QTL region on LG X, another gene (estExt_Genewise1_v1.1_LG_X3745) involved in ethylene response also showed significant divergence in response between the extreme genotypes and the two grandparental species. This ethylene response factor was down-regulated (2 fold) in both *P. deltoides* and the tolerant extreme genotypes but was not differentially expressed in *P. trichocarpa* and was up-regulated in the sensitive genotypes. The direction of differential expression between both the grandparents and the sensitivity genotypes is particularly interesting considering the role of ethylene in ozone response shown in Overmyer et al. (2005) and Tamaoki et al. (2003a,b). Low-level ethylene production triggered stress responses more similar to pathogen induced responses while higher levels of ethylene production trigger lesion formation and propagation, a finding that is consistent with the expression changes observed in this study. A significant hotspot of co-locating QTL was found on LG X for early and late season leaf number, height.

Fig. 2. QTL for ozone-associated physiological traits. QTL are plotted as a CI defined as an F2 drop off with peak F score location being marked with a short horizontal mark. The genetic linkage group for Family 331 is plotted (grey). cM locations of markers and marker names are shown for SSR (dark blue, black text) and AFLP (light blue, grey text). Control (blue), percentage effect response to ozone (green) and ozone exposure (red) QTL are plotted, with QTL explaining >5% trait variation plotted in bold. Orange lines represent region over-represented with co-locating QTLs, identified using a sliding window approach. The chromosome (pale blue bar) is shown with SSR locations marked as blue horizontal lines and candidate genes as black lines with their associated abbreviated name. Dotted lines connect SSRs between the linkage map and chromosome where possible. Candidate genes are those that were most informative in explaining the difference in response to ozone between the tolerant and sensitive F2 genotypes from microarray data (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).
Table 6

<table>
<thead>
<tr>
<th>Gene Model</th>
<th>Protein ID</th>
<th>Log2 fold change</th>
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<th>LG</th>
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<td>AT5G04160 XII ATOMT1 (O-METHYLTRANSFERASE 1)</td>
</tr>
</tbody>
</table>

and diameter. This hotspot co-locates to three genes that were differentially expressed between the two sensitivity groups; a chitinase gene, BG1 (BETA-1,3-GLUCANASE 1), and stable protein 1.

4. Conclusion

Ozone greatly increased the occurrence of visible damage, manifested as small flecks or larger necrotic spots, as observed in a range of species (Berrang et al., 1991; Heggestad and Middleton, 1959; Kargioliaki et al., 1991; Piikki et al., 2004). There was considerable variation in the extent of this damage, with some genotypes showing very little damage. In contrast, some genotypes had over 90% damaged leaves later in the season. The marked increase in leaf abscission is consistent with previous work (Bohler et al., 2007; Karnosky et al., 1996; Woo and Hinckley, 2005), and serves to demonstrate the detrimental effect of ozone on leaf biomass. The treatment specific co-location of QTL for leaf number, leaf abscission and visible leaf damage with basal diameter suggests that these may be the responses resulting in reduced leaf abscission and visible leaf damage with basal diameter. This hotspot co-locates to three genes that were being ozone-responsive, evidenced here are also more generally involved in abiotic stress responses. This suggests that at the global scale, induced gene responses are conserved across species and that there appears to be a conserved general abiotic stress-induced remodelling of the transcriptome, in response to a variety of stresses. Genes encoding enzymes involved in the phenylpropanoid pathway were up-regulated as a result of ozone exposure, as was found in similar studies in a range of species (Koch et al., 2000; Ludwikow et al., 2004; Matsuyama et al., 2002; Puckette et al., 2008). Re-modelling of secondary metabolism is apparently an important and conserved mechanism of response to ozone exposure, and is a response that will divert energy from primary metabolism. This has potential downstream effects on productivity, in accordance with the growth difference balance hypothesis extended by Hermas and Matson (1992). Although it is evident that changing growth environments between controlled chambers and field-conditions has a bigger impact on transcriptome response for the model plant A. thaliana than does exposure to either increased ozone or CO2 (Miyazaki et al., 2004). Here differential gene expression was examined following acute ozone exposure in the indoor controlled environments while QTL were identified following chronic ozone exposure in the chambered field study. Although such out-door chambers do not reflect the true field conditions (Hendrey and Miglietta, 2006) this combination of approaches and an observed consistency between the selected genotypes within each experimental conditions provides a robust insight into transcriptome responses of Populus to tropospheric ozone.

In summary we have linked genetics and genomics to increase understanding of the control of adaptive responses to ozone in two Populus species and groups of F2 genotypes that exhibit divergent sensitivities to acute and chronic ozone treatment. QTL mapping identified regions of the genome involved in trait expression, including those specific to ozone stressed plants. Genes such as SAM synthetase, a chitinase gene, Beta-1,3-Glucanase and Stable protein 1 exhibited expression differences between sensitivity groups and were found to co-localise to QTL for necrotic damage, providing encouraging evidence for their importance in governing this trait.

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Appendix. Supplemental material

Supplemental material associated with this article can be found in the online version, at doi:10.1016/j.envpol.2010.09.027.

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