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The physiological, transcriptional and genetic responses of an ozone- sensitive and an ozone tolerant poplar and selected extremes of their F_2 progeny

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

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ABSTRACT

Relatively little is known about the transcriptional response or genetic control of response and adaptation of trees to tropospheric ozone exposure. Such understanding is needed as up to 50% of forests, globally, may be subjected to phytotoxic concentrations of ozone. The physiological, transcriptional and genetic response to ozone was examined in *Populus trichocarpa* and *P. deltoides*, which show extreme sensitivity and tolerance to ozone, respectively. Using an inbred F_2 mapping population derived from these two species, we mapped quantitative trait loci (QTL) for traits associated with ozone response, examined segregation of the transcriptional response to ozone and co-located genes showing divergent responses between tolerant and sensitive genotypes with QTL. QTL were identified linking detrimental effects of ozone with leaf and biomass traits and differential responses were found for key genes involved in ethylene production and response.

1. Introduction

Tropospheric ozone concentrations have increased by ~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; Wittig et al., 2009). Karnosky et al. (2005) reported the long-term effects of ozone pollution on several tree species, and demonstrated a negative impact throughout the whole ecosystem, not only on the trees themselves but also on associated pests and soil microorganisms.

Stomata regulate the entry of ozone into the leaf (Vahisalu et al., 2008), and when ozone reaches the apoplast, production of toxic reactive oxygen species (ROS) is initiated (Mahalingam et al., 2006; Wohlgemuth et al., 2002). Symptoms range from accelerated senescence (Karnosky et al., 1996) and a decrease in growth rate (Karnosky et al., 1996, 2005; Wittig et al., 2009), to foliar injury manifested as necrotic lesions analogous to those formed during the hypersensitive response (HR) (Langebartels et al., 2002). Concomitant with these symptoms is an up-regulation of defence and stress related genes and compounds (Conklin and Last, 1995; Lindroth, 2010; Ludwikow et al., 2004), and a down-regulation of photosynthetic components (Conklin and Last, 1995; Glick et al., 1995). Although transcriptional responses to ozone and underlying QTL have been studied in annual model and non-model species (Frei et al., 2008; Li et al., 2006; Overmyer et al., 2008; Puckette et al., 2008), there is currently limited knowledge in perennials, such as trees, which form the keystone species of many terrestrial ecosystems.

Populus has 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).

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Here, we examined the genetic and genomic responses of *Populus* to chronic and acute ozone exposure. We investigated the genetics and genomics of response to ozone in two divergent *Populus* species and their inbred F_2 progeny in two experiments. In the first chambered field study, sensitive and tolerant ozone genotypes of the F_2 population were identified and QTL for ozone response determined. These were studied in more detail, alongside the grandparental species in controlled environments using a transcriptomics approach.

2. Materials and methods

2.1. Chronic ozone exposure

2.1.1. Growth chambers and ozone regime

The experiment was conducted in 16 glass chambers at the Forest Research closed nursery site at Headley, Hampshire, UK (described in Broadmeadow et al., 1999; Broadmeadow and Jackson, 2000). Eight chambers were ozone enriched, and eight exposed to ambient air. Prior to planting, dolomitic lime, base dressing and slow release Osmocote (15:9:12) fertiliser were applied at the recommended dose. Plants were irrigated daily by overhead sprinklers ($\sim$12 L h^{-1} for 30 min from 07:00–07:30, and from 19:00–19:30). Airflow was approximately 0.57 m^3 s^{-1} in all chambers, equating to approximately two air changes min^{-1} reduced to approximately 0.28 m^3 s^{-1} between 20:00 and 10:00. Day length was not controlled. Ozone was generated by electrical discharge, using a model B063 Odotrol generator (Wallace & Tiernan, Kent, UK) and ozone was passed through a water trap to remove N_2O_5 (Broadmeadow and Jackson, 2000). Ozone concentration was measured optically (model 8810, Monitor Labs, California, USA). Needle valves were used to balance the flow to each chamber. The exposure regime consisted of a square waveform fumigation with ozone exposure occurring between 11:00–18:00 from 2004-07-14–2004-10-20. Mean ozone concentration in treatment chambers were 93.1 nL L^{-1} during fumigation and was 7.9 nL L^{-1} outside the fumigation period (*i.e.* at night). Mean ozone concentration in control chambers was 16.7 nL L^{-1} during fumigation, and 7.9 nL L^{-1} outside the fumigation period. Within treatment chamber variability was tested by ANOVA no significant difference was found (data not shown).

2.1.2. Plant material

An inbred F_2 mapping population (Family 331), formed from a cross between *Populus trichocarpa* Torr. & Gray (93–968) and *P. deltoides* Bart (ILL-129), known to be highly divergent for a vast range of phenotypic traits was developed by Bradshaw et al. (1994). This pedigree was imported into the UK in 1999 (see Rae et al., 2009 for details). Hardwood cuttings used in this study were obtained from a field site in Hampshire, UK (see Rae et al., 2004 for details). Cuttings of uniform length and that were of uniform diameter within each genotype (*i.e.* cutting diameter *between* genotypes was as consistent as possible but may vary but *within* genotype all diameters were uniform) were stored at 2 °C until required. Three replicates of 164 F_2 genotypes and the *P. trichocarpa* and *P. deltoides* grandparents were each assigned within the eight chambers ensuring that replicates of each genotype were distributed evenly across the chambers. The pattern was repeated for the ambient and treated groups. Cuttings were soaked in cold water for 24 h prior to planting and were planted on 2004-06-04 in rows 25 cm apart with two viable buds above the soil surface. Plants were sprayed with Ambush on 2004-06-22 to control beetle damage, and with Cypermethrin on 2004-09-24 for spider mite. Plants were grown for 33 days before the ozone treatment was initiated.

2.1.3. Acute ozone exposure

Described in Supplementary materials and methods.

2.1.4. Trait measurements

Described in Supplementary materials and methods.

2.1.5. Data analysis

Data were analysed using GLM-ANOVA models in Minitab 14 (Minitab, Coventry, UK) and where necessary data were normalised using a Box–Cox transformation (Box and Cox, 1964). An initial ANOVA was performed to test for chamber and position within chambers effects. As these factors were not significant, they were eliminated from the model. Genotype, treatment and interaction effects were tested using the model (5)

$$Y_{ijk} = \mu + G_i + T_j + G \times T_{ij} + \varepsilon_{ijk} \quad (5)$$

where μ is the general mean, G_i is the genotype effect (random), T_j is the treatment effect (fixed), $G \times T_{ij}$ is the genotype by treatment interaction and ϵ_{ijk} is the error.

2.1.6. QTL mapping

All genotypes used for QTL mapping were full-sib progeny (referred to here as the F_2 generation) of Family 331. QTL were mapped using the freely available web-based program QTLExpress (Seaton et al., 2002) using the out-breeding module. Permutation testing implemented in QTLExpress was used to establish the critical F value for declaring a QTL present (1000 permutation, see Churchill and Doerge, 1994). QTL confidence intervals (CIs) were calculated using an F-two drop-off (the cM distance taken for the peak *F* value to drop by two). The genetic linkage map used was produced by Tuskan et al. (pers. comm.) and consists of 91 SSR markers genotyped on 350 of the full-sib progeny and 92 fully informative Amplified Fragment Length Polymorphisms (AFLPs) genotyped on 165 genotypes of the progeny. The resulting genetic map consists of 22 Linkage Groups (LG). Where more than one LG has been assigned to a chromosome, they are numbered with the LG number and a letter, letter order indicates the order of LGs along the chromosome. SSR primer sequences (http://www.ornl.gov/sci/ipgc/ssr_resource.htm) were located on the genome sequence to align the genetic and physical maps and to provide correct orientation of linkage groups (i.e. 3′ to 5′). The location information of SSR markers was used to generate gene lists of all genes between flanking SSR markers of a subset of QTL. QTL figures were produced using a custom-written R package developed by ourselves and available on request. This package implements a permutation test and sliding window approach to identify regions of the genetic map over-represented with co-locating QTL (See Kliebenstein et al., 2006). For each permutation, QTL are randomly shuffled across the genome and a sliding window of 5 cM is then used to count the number of QTL in each window region. The window was advanced in 1 cM steps across the entire genetic map and the maximum number of QTL in a window region was recorded per permutation. The permutation maximum count results were then sorted and used to determine the critical value at an α0.05 significance level (the 950th value for 1000 permutations). The sliding window was then applied to the original QTL data to identify regions with more than the critical number of co-locating QTL. The critical number for our data was five (1000 permutations).

2.2. Transcriptional response to acute ozone

2.2.1. P. trichocarpa and P. deltoides grand parental species

After nine hrs of ozone exposure, one plant of each species was sampled from each chamber, with an additional *P. trichocarpa* sample taken from a randomly chosen chamber, giving a total of five replicates for *P. trichocarpa* and four for *P. deltoides* for both treated and control. The fifth leaf below the first fully unfurled leaf was frozen in liquid N_2 for RNA extraction. Total RNA was extracted as described in Street et al. (2006). RNA concentration was determined using an Eppendorf Biophotometer (Eppendorf, Hamburg, Germany), and quality was assessed using a 1% (w/v) agarose gel.

The microarrays used are described in Brosché et al. (2005) and were performed as described there. The constructed cDNA libraries were derived from *P. euphratica* grown in Ein Advat Valley, Israel, and also from control and stress exposed trees. From these libraries, 8153 ESTs were spotted on poly-lysine slides (Brosché et al., 2005).

2.2.2. Analysis of sensitive and tolerant genotypes

Leaf sampling was carried out as described above. For each sampled leaf a digital image was taken and the percentage coverage of ozone-induced necrosis was estimated.

RNA was extracted as described above. Equal amounts of RNA from the five replicates of each genotype for each treatment were pooled to give 100 μg total RNA per treatment and genotype to be used for cDNA synthesis. One slide was hybridised per genotype with each slide co-hybridising the control and treated samples for that genotype. This experimental design gives a replication of three for each ozone response group (one genotype represents one biological replicate), with each of those three replicates representing a pool. As our intention was to test for differences between the two ozone response groups, we felt that this pooling strategy was suitable and still maintained replication at the comparison level of interest (*i.e.* tolerant vs. sensitive). However, we acknowledge that our results only infer differences at this level.

The poplar PICME microarray (www.picme.at) consisting of 26,915 ESTs including all those used for the grandparental acute ozone study was used for this experiment. The ESTs printed on the PICME poplar arrays were produced by INRA-Nancy (Rinaldi et al., 2007), INRA-Orleans (Déjardin et al., 2004), and University of Helsinki (Brosché et al., 2005) within the framework of the LIGNOME and ESTABLISH programme respectively. Full MIAME-compliant details of the array content and production can be found at www.picme.at (also see below). Probes were prepared and labelled as for the grandparental experiment (see Brosché et al., 2005 for details). All array data generated has been deposited in the Gene Expression Omnibus (GEO) database as superSeries GSE10932. Full hybridisation and analysis methods are available as part of this submission.

2.2.3. Microarray data analysis

Intensity values were print-tip LOWESS normalised to control for within-array signal intensity artefacts and cross-slide normalisation was also applied to ensure consistency of scale. See Smyth and Speed (2003) for a review of microarray normalisation. Differentially expressed ESTs between control and ozone-exposed treatments were identified using the 'B statistics' as described in Tallis et al. (2010).

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

The list of significantly differentiated 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* 'Nisqually-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. 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 time point. 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*.

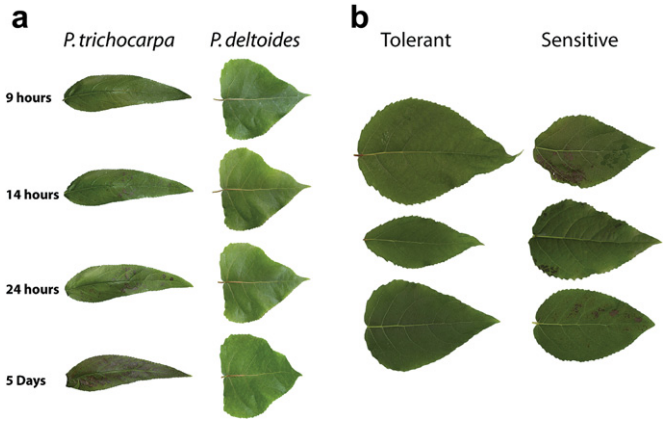

Fig. 1. a The progression of ozone damage for *P. deltoides* and *P. trichocarpa* exposed to 200 nL L^{-1} ozone in growth chambers for 5 days, showing necrotic lesion formation in *P. trichocarpa*, with a lack of visible symptoms in *P. deltoides*. b The response of three ozone tolerant and three sensitive 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 ($F_{1,132} = 11.24$, $p < 0.001$) 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 ($F_{1,126} = 5.45$, $p < 0.05$) 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*treatment interaction, suggesting that differential response mechanisms exist within the F_2 genotypes (Table 2).

Table 1
Summary of traits measured in *P. deltoides* and *P. trichocarpa* 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	P. deltoides				*P. trichocarpa*			
	Control	Ozone	% Effect	Trt	Control	Ozone	% Effect	Trt
Height (cm)	55.30	53.00	−4.34	ns	79.30	72.30	−9.68	ns
Diameter (mm)	6.03	5.43	−10.00	ns	6.47	5.17	−20.20	*
Leaf number (30d)	15.70	14.70	−6.80	ns	19.70	15.30	−28.76	ns
Leaf number (70d)	21.30	18.30	−16.39	*	26.70	26.00	−2.69	ns
Chlorophyll content	15.10	9.53	−58.45	**	20.70	21.90	5.48	ns
% Abscised (30d)	7.10	17.60	59.66	***	8.80	10.30	14.56	*
% Abscised (70d)	18.50	40.00	53.75	***	22.80	53.90	57.70	***
Area of first unfurled leaf (cm)	7.40	6.64	−11.38	*	9.94	12.62	21.20	*
Leaf expansion rate (% increase)	74.60	68.20	−9.38	ns	87.20	54.40	−60.29	*

Table 2
Summary of traits measured in the F_2 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Control	Ozone	% effect	Trt	Geno	Geno*Trt
Height (cm)	78.70	79.74	1.30	ns	***	ns
Diameter (mm)	7.09	6.57	−3.20	***	***	ns
Leaf number (30d)	19.60	20.30	3.44	*	***	ns
Leaf number (70d)	26.10	27.15	3.84	**	***	ns
Chlorophyll content	21.06	21.22	0.78	ns	***	*
% abscised (30d)	2.45	12.77	80.78	***	***	NA
% abscised (70d)	14.59	33.24	56.11	***	***	*
Area of first unfurled leaf (cm)	10.56	11.81	10.60	*	***	ns
Leaf expansion rate (% increase)	99.80	90.70	−10.03	**	*	ns

3.3. QTL mapping in the F_2 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 (Utz 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.

Of the 58 QTL detected, 20 were found to map only under ozone treatment or as response QTL (indicated in bold in Table 3). It is likely that these represent genomic regions that are specifically involved in governing the response to ozone, and are the most encouraging QTL for further investigation. Such QTL were found for leaf necrosis, diameter, late-season leaf number, height, late season abscission, area of the first unfurled leaf and chlorophyll content, indicating that ozone responsive genomic regions exist that govern numerous traits.

A number of ozone stress responsive loci were mapped with co-locating QTL for more than one trait, suggesting that these loci control adaptive response mechanisms that impact the general physiology of the plant. LGs III, IV, Vb, VI, X and XI all contain such examples. Three examples show co-location to QTL for basal diameter that were mapped only in the ozone treatment or as response (percentage response to ozone) QTL (Table 3). These loci warrant future attention as they appear to be those most likely to control the reduced diameter growth in response to ozone exposure.

3.4. Sensitive and tolerant F_2 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 F_2 genotypes at the population extremes for the trait of interest (visible 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 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). 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 the QTL were only mapped in T and/or R.

Trait	C/T/R	LG	Position (cM)	CI	p value	%Vp
Area of first unfurled leaf	C	VIIIa	0	0–4	0.006	12.36
	C	XV	44.2	34.2–61.2	0.029	10.15
	R	XIX	13.7	0–32.7	0.024	9.19
Basal stem diameter	C	X	0.2	0–13.2	0.004	11.4
	T	III	67.2	47.2–86.2	0.018	2.32
	T	IV	84.8	78.8–84.8	0.003	3.02
	T	Vb	0	0–19.3	0.044	1.51
	T	X	33.2	0–51.2	0.038	7.9
	T	XII	23	0–29	0.003	2.8
Chlorophyll content	C	I	82	64–96	0.0011	15
	C	VI	7.2	0–21.2	0.004	12.75
	C	VIIIb	14	9–25.3	0.001	11.56
	C	XIV	0.6	0–14.6	0.019	7.73
	R	Vb	0	0–19.3	0.026	6.81
	R	XI	55	29–55.6	0.042	7.3
	R	XII	0	4.–24.6	0.001	12.88
	R	XVII	53	64–68	0.017	9.3
	T	VI	139.2	132.2–143.2	0.043	9.1
	T	VIIIb	3	0–11	0.004	11.13
	T	X	29.2	8.2–49.2	0.03	8.57
	T	XI	36	18–55.6	0.048	7.45
Height	C	X	0.2	0–9.2	0.004	11.3
	C	XV	40.2	36.2–50.2	0.005	10.95
	R	VI	95.2	82.2–112.2	0.003	12.6
	T	X	0.2	0–12.2	0.043	7.23
	T	XII	24	14.24.6	0.05	6.48
	T	XV	49.2	35.2–61.2	0.044	7
Leaf abscission (30d)	C	II	82	40–103	0.032	8.7
	T	VI	101.2	69.2–118.2	0.013	10.78
	T	X	52.2	29.2–62.2	0.03	8.68
Leaf abscission (70d)	C	X	38.2	26.2–54.2	0.011	11.24
	C	XIX	1.7	0–13.7	0.007	11.77
	R	IV	6.8	0–25.8	0.016	11.12
	T	X	62.2	54.2–62.2	0.021	9.74
	T	XIII	72	72.88	0.05	9.11
Leaf expansion rate	C	XVII	38	32–58	0.014	11.95
	R	XVII	47	33–66	0.02	11.18
	T	XV	40.2	0–52.2	0.02	9.48
Leaf number (30d)	C	X	3.2	0–34.2	0.032	9.33
	R	VI	95.2	8.6–108.2	0.043	9.27
	T	X	7.2	0–23.2	0.019	9.48
Leaf number (70d)	C	X	0.2	0–35.2	0.004	11.64
	R	VI	95.2	86.2–105.2	0.012	13.15
	R	VIIIb	18	5–25.3	0.027	5.66
	R	XI	52	43–55.6	0.005	9.56
	T	III	69.2	48.2–86.2	0.016	9.73
	T	Va	48	32–72	0.01	10.4
	T	VI	109.2	95.2–119.2	0.029	10.71
	T	X	8.2	0–44.2	0.015	10.05
Visible damage (30d)	**T**	II	112	102–113.7	0.036	7.9
	T	XVIII	61.7	45.7–84.7	0.037	6.98
Visible damage (70d)	**T**	I	117	108–122	0.001	12.65
	T	II	105	86.109	0.013	10.16
	T	IV	84.8	71.8–84.8	0.001	13.02
	T	IX	9	0–9.3	0.005	8.84
	T	VI	0.2	0–17.2	0.011	10.09
	T	VIIIa	27	17–27.1	0.044	6.51
	T	X	62.2	47.2–62.2	<.0001	16.33

Table 4
Means of physiological traits for the selected sensitive and tolerant clones of Family 331, identified from a 100 nL L^{-1} ozone exposure in open top chambers. For traits measured on more than one occasion, this is indicated by 30d (30 days of exposure) and 70d (70 days of exposure). Results of ANOVA are given, with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. G = genotype, T = treatment, S = sensitivity group, TS = interaction between treatment and sensitivity group, TG = interaction between treatment and genotype.

	Control		Ozone		G	T	S	TS	TG
	Tolerant	Sensitive	Tolerant	Sensitive					
% Damaged 30d	–	–	1.36	19.54	–	–	*	–	–
% Damaged 70d	–	–	24.05	40	–	–	**	–	–
% Abscised 30d	0.53	2.89	11.26	14.08	ns	*	ns	ns	**
% Abscised 70d	11.84	10.11	16.08	29.25	ns	ns	ns	ns	**
% Necrotic	–	–	0.18	23.23	–	–	**	–	–
Height (cm)	88.33	78.58	77.33	88.28	ns	ns	ns	ns	ns
Diameter (mm)	8	6.94	6.53	6.97	ns	ns	ns	ns	ns
Chlorophyll content	20.34	20.58	24.37	33.34	*	*	ns	ns	ns
Leaf area 1 (cm^2)	9.52	6.4	7.91	12.34	**	**	ns	***	ns
Leaf area 2 (cm^2)	22.21	13.31	14.14	21.8	*	ns	ns	**	ns
% area increase	134	116	81	84	ns	*	ns	ns	ns
Leaf number 30d	23.45	21	20.5	23.87	ns	ns	ns	ns	ns
Leaf number 70d	30.81	28	29.41	32.2	ns	ns	ns	ns	ns

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 F_2 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 F_2 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 638 were down-regulated, with $\log_2$ 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 carbohydrate metabolism. The most represented up-regulated categories were those involved in biotic and abiotic stress responses, and secondary metabolism. Table 5 provides details of the 50 most significantly differentially expressed genes in response to ozone.

Analysis of control-treatment response in common to both F_2 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 $\log_2$ 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 ($\log_2$ 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 F_2 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 F_2 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 that 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.

Table 5
50 most significant differentially expressed genes in common to *P. deltoides* and *P. trichocarpa* after exposure to 9 hrs 200 nL L^{-1} ozone. The average expression across both species is given. See Table S1 for further details and the significance of all genes on the microarray. Gene model and Protein ID refer to the JGI v1.1 Jamboree gene model identifiers. ATG codes identify *Arabidopsis* orthologs, inferred by best-hit BLAST searches of poplar protein against *Arabidopsis* protein sequences. Short descriptions are those for *Arabidopsis* orthologs contained in TAIR 7.

Gene Model	Protein ID	ATG	Log_2 fold change	Adj. *P*	Short description
gw1.5570.1.1	290965	AT1G76690	−2.12	0.00018	OPR2 (12-oxophytodienoate reductase 2)
estExt_Genewise1_v1.C_1460016	745223	AT3G14940	−2.11	0.00034	ATPPC3 (PHOSPHOENOLPYRUVATE CARBOXYLASE 3)
estExt_fgenesh4_pm.C_1230010	837012	AT3G02360	−2.17	0.00035	6-phosphogluconate dehydrogenase family protein
eugene3.00040033	555320	AT4G04640	2.01	0.00041	ATPC1 (ATP synthase gamma chain 1)
estExt_Genewise1_v1.C_LG_XIV0740	730849	AT2G45290	1.33	0.00042	transketolase, putative
estExt_Genewise1_v1.C_LG_XI0270	726993	AT4G27270	−3.39	0.00045	quinone reductase family protein
estExt_fgenesh4_pg.C_1330041	828033	AT5G27760	−2.65	0.00047	hypoxia-responsive family protein
estExt_fgenesh4_pg.C_LG_X1392	822254	AT3G08900	−1.79	0.00053	RGP3 (REVERSIBLY GLYCOSYLATED POLYPEPTIDE 3)
estExt_fgenesh4_pg.C_LG_IX0475	821174	AT2G21620	−1.82	0.00053	RD2 (RESPONSIVE TO DESSICATION 2)
grail3.0189002602	663639	AT1G26910	−1.74	0.00053	60S ribosomal protein L10 (RPL10B)
eugene3.00011774	549333	AT5G11740	−1.56	0.00053	AGP15 (ARABINOGALACTAN PROTEIN 15)
fgenesh4_pg.C_scaffold_29000012	796254	AT1G72310	−2.12	0.00053	ATL3 (Arabidopsis T?xicos en Levadura 3)
grail3.0155001302	673066	AT5G15650	−2.62	0.00053	RGP2 (Reversibly glycosylated polypeptide)
gw1.118.151.1	264663	AT4G17030	−2.50	0.00053	ATEXLB1 (ARABIDOPSIS THALIANA EXPANSIN-LIKE B1)
grail3.0025024002	668125	AT5G01650	−1.80	0.00053	macrophage migration inhibitory factor family protein
estExt_fgenesh4_pm.C_LG_XII0373	834364	AT4G24990	−2.08	0.00053	ATGP4 (Arabidopsis thaliana geranylgeranylated protein)
eugene3.00110215	568201	AT5G54770	−1.53	0.00067	THI1 (THIAZOLE REQUIRING)
eugene3.00181016	578792	AT4G31985	−1.90	0.00067	60S ribosomal protein L39 (RPL39C)
estExt_fgenesh4_pg.C_21210001	829083	AT1G14870	−2.01	0.00067	
eugene3.00090275	557015	AT3G12500	−1.36	0.00067	ATHCHIB (BASIC CHITINASE); chitinase
estExt_Genewise1_v1.C_LG_II1841	710544	AT4G02380	2.56	0.00067	SAG21 (SENESCENCE-ASSOCIATED GENE 21)
gw1.XII.485.1	422025	AT3G22370	−2.22	0.00068	AOX1A (alternative oxidase 1A); alternative oxidase
estExt_Genewise1_v1.C_1970084	746640	AT5G24090	−3.42	0.00071	acidic endochitinase (CHIB1)
gw1.XIV.108.1	243365	AT5G43940	−1.76	0.00080	ADH2 (ALCOHOL DEHYDROGENASE 2)
estExt_fgenesh4_pg.C_LG_I2142	815507	AT3G27820	−2.31	0.00083	ATMDAR4 (MONODEHYDROASCORBATE REDUCTASE 4)
gw1.X.2853.1	228156	AT3G10420	−2.37	0.00083	sporulation protein-related
estExt_fgenesh4_pg.C_LG_XV0035	824484	AT5G54160	−1.44	0.00083	ATOMT1 (O-METHYLTRANSFERASE 1)
estExt_Genewise1_v1.C_LG_X0765	724015	AT1G02500	1.38	0.00083	SAM1 (S-adenosylmethionine synthetase 1)
estExt_fgenesh4_pg.C_LG_IV0366	817806	AT1G29290	−1.90	0.00083	
gw1.IX.2337.1	201872	AT4G34200	−3.40	0.00088	EDA9 (embryo sac development arrest 9);
fgenesh4_pg.C_scaffold_3758000001	792358	AT1G58170	−1.82	0.00088	disease resistance-responsive protein-related
eugene3.00100340	565782	AT2G33470	2.18	0.00096	GLTP1 (GLYCOLIPID TRANSFER PROTEIN 1)
gw1.II.1221.1	409886	AT4G01150	−2.26	0.00096	
gw1.131.45.1	268043	AT3G62020	−1.78	0.00096	GLP10 (GERMIN-LIKE PROTEIN 10)
estExt_Genewise1_v1.C_LG_III0933	712009	AT3G24170	−1.37	0.00096	ATGR1; glutathione-disulfide reductase
estExt_fgenesh4_pm.C_LG_XIV0249	834831	AT2G47000	1.79	0.00096	PGP4 (P-GLYCOPROTEIN 4, P-GLYCOPROTEIN4)
gw1.XIV.3318.1	246575	AT2G05710	−1.31	0.00097	aconitate hydratase, cytoplasmic, putative/citrate hydro-lyase/aconitase, putative
estExt_fgenesh4_pg.C_LG_VI0690	819178	AT5G02160	−3.15	0.00100	
estExt_Genewise1_v1.C_LG_III0308	711753	AT1G64160	1.68	0.00100	disease resistance-responsive family protein
gw1.182.27.1	276236	AT1G08170	−2.13	0.00102	histone H2B family protein
gw1.IV.3246.1	198157	AT1G67090	2.38	0.00106	ribulose bisphosphate carboxylase small chain 1A
estExt_fgenesh4_pg.C_LG_V1224	818640	AT1G06680	2.01	0.00106	PSBP-1 (OXYGEN-EVOLVING ENHANCER PROTEIN 2)
fgenesh4_pm.C_LG_XVI000502	808774	AT1G23740	−2.17	0.00106	oxidoreductase, zinc-binding dehydrogenase family protein
fgenesh4_pm.C_LG_IX000091	804044	AT5G22770	2.54	0.00106	ALPHA-ADR (ALPHA-ADAPTIN)
estExt_Genewise1_v1.C_LG_XI1233	727425	AT3G21690	−1.61	0.00106	MATE efflux family protein
estExt_fgenesh4_pg.C_LG_IV0222	817755	AT4G05020	−1.93	0.00106	NDB2 (NAD(P)H DEHYDROGENASE B2); disulfide oxidoreductase
eugene3.00160400	576265	AT1G24020	−2.66	0.00106	Bet v I allergen family protein
estExt_fgenesh4_pg.C_LG_XIV1000	824336	AT5G14780	−1.43	0.00106	FDH (FORMATE DEHYDROGENASE)
gw1.XVIII.2487.1	261946	AT4G29060	1.26	0.00111	EMB2726 (EMBRYO DEFECTIVE 2726); translation elongation factor
grail3.0018023401	646534	AT4G22920	2.05	0.00126	

Three genes co-located to a QTL for total damage (end-season damage) on LG I. These genes code for UDP-xyl synthase 5 (USX5), 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 (Harper and Bar-Peled, 2002). 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). Arabinogalactans have also been shown to be highly expressed in tension wood in poplar (Lafarguette 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; Pandey et al., 2000), 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 polyamines 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

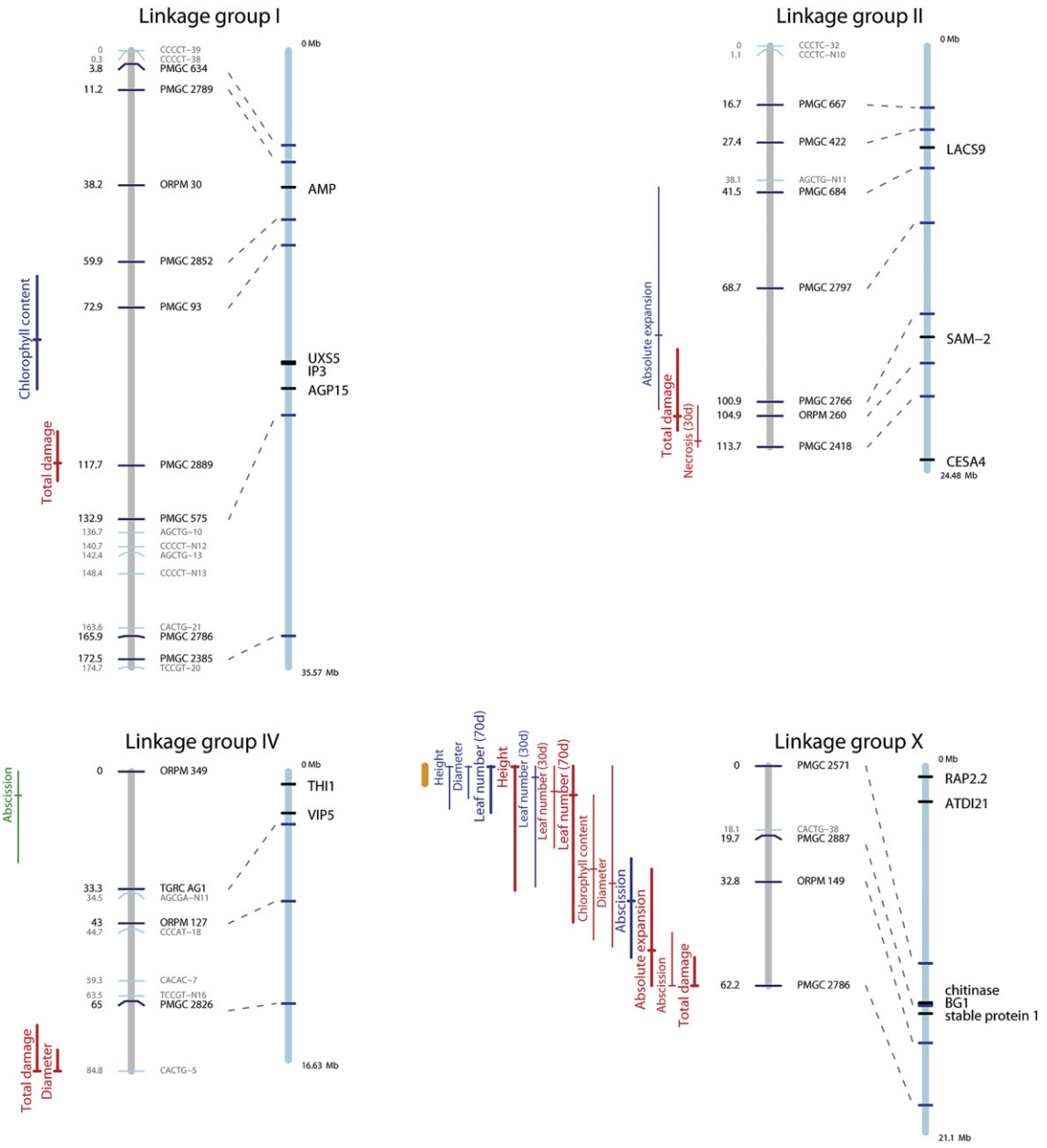

Fig. 2. QTL for ozone-associated physiological traits. QTL are plotted ± CI defined as an F2 drop off with peak F score location being marked with a short horizontal mark. The genetic linkage group for Family 331 is plotted (grey), cM locations of markers and marker names are shown for SSR (dark blue, 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 F_2 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.).

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.C_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

Table 6
Genes that were informative in explaining the separation of the transcriptional responses of the sensitive and tolerant genotypes, as identified using PLS-DA, and that showed co-location to mapped QTLs. Gene model and Protein ID refer to the JGI v1.1 Jamboree gene model identifiers. ATG codes identify *Arabidopsis* orthologs, inferred by best-hit BLAST searches of poplar protein against *Arabidopsis* protein sequences. Short descriptions are those for *Arabidopsis* orthologs contained in TAIR 7.

Gene Model	Protein ID	Log2 fold change		ATG	LG	Short description
		Tolerant	Sensitive			
estExt_Genewise1_v1.C_LG_I1353	706198	0.39	0.02	AT3G46440	I	UXS5 (UDP-Xyl synthase 5); catalytic
eugene3.00011653	549212	−0.71	2.45	AT4G01470	I	GAMMA-TIP3/TIP1;3 (tonoplast intrinsic protein 1;3)
eugene3.00011774	549333	0.75	2.47	AT5G11740	I	AGP15 (ARABINOGALACTAN PROTEIN 15)
grail3.0050014702	644907	2.05	0.06	AT4G01850	II	MAT2/SAM-2 (S-adenosylmethionine synthetase 2)
estExt_fgenesh4_pg.C_LG_IV0080	817727	−0.07	−3.68	AT5G54770	IV	THI1 (THIAZOLE REQUIRING)
eugene3.00040262	555549	5.73	1.93	AT1G61040	IV	VIP5 (VERNALIZATION INDEPENDENCE 5)
gw1.VI.1805.1	417432	−0.41	1.99	AT3G22120	VI	CWLP (CELL WALL-PLASMA MEMBRANE LINKER PROTEIN)
estExt_fgenesh4_pg.C_LG_VIII1530	820835	5.80	3.82	AT2G29420	VIII	ATGSTU7 (GLUTATHIONE S-TRANSFERASE 25)
estExt_Genewise1_v1.C_LG_X3745	725612	−2.03	2.46	AT3G14230	X	RAP2.2; DNA binding/transcription factor
estExt_fgenesh4_pg.C_LG_X0149	821723	−1.34	1.29	AT4G15910	X	ATDI21 (Arabidopsis thaliana drought-induced 21)
estExt_Genewise1_v1.C_LG_X0543	723883	0.01	−1.34	AT3G16920	X	chitinase
fgenesh4_pg.C_LG_X001297	769807	1.46	4.82	AT3G57270	X	BG1 (BETA-1,3-GLUCANASE 1
estExt_fgenesh4_pm.C_LG_X0585	833676	−0.87	−1.79	AT3G17210	X	stable protein 1-related
estExt_fgenesh4_pm.C_LG_XII0129	834247	1.43	0.97	AT5G54160	XII	ATOMT1 (O-METHYLTRANSFERASE 1)

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; Kargiolaki 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 diameter growth. Such an understanding of the genetic control leading to reduced biomass production is extremely useful for directing future research directions and breeding approaches. For example, Wittig et al. (2009) suggest that relative to preindustrial levels, current tropospheric ozone has already reduced Northern temperate and boreal forest productivity by 7%, while future predictions suggest an 11% reduction by 2050 (Wittig et al., 2009). Concurrent to this, atmospheric carbon dioxide concentrations ($[CO_2]$) have increased ~ 40% since preindustrial times and Karnosky et al. (2003) identified that for *Populus* at least, such future biomass reductions by ozone will be mitigated by increased atmospheric $[CO_2]$.

Many of the differentially expressed genes identified here as being ozone-responsive, confirm previous studies on other species (Gupta et al., 2005; Li et al., 2006; Matsuyama et al., 2002; Puckette et al., 2008). Genes identified 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 down-stream effects on productivity, in accordance with the growth difference balance hypothesis extended by Herms and Mattson (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 CO_2 (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 F_2 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. Supplementary material

Supplementary 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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