Partial correlation analysis of transcriptomes helps detangle the growth and defense network in spruce

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[†] In memory of Rick White (1964-2016)

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SUMMARY

- In plants, there can be a trade-off between resource allocations to growth versus defense. Here, we use partial correlation analysis of gene expression to make inferences about the nature of this interaction.
- We studied segregating progenies of Interior spruce subject to weevil attack. In a controlled experiment, we measured pre-attack plant growth and post-attack damage with several morphological measures, and profiled transcriptomes of 188 progeny.
- We used partial correlations of individual transcripts (ESTs) with pairs of growth/defense traits to identify important nodes and edges in the inferred underlying gene network, e.g., those pairs of growth/defense traits with high mutual correlation with a single EST transcript. We give a method to identify such ESTs.
- A terpenoid ABC transporter gene showed strongest correlations (P=0.019); its transcript represented a hub within the compact 166-member gene-gene interaction network (P=0.004) of the *negative* genetic correlations between growth and subsequent pest attack. A small 21-member interaction network (P=0.004) represented the uncovered *positive* correlations.
- Our study demonstrates partial correlation analysis identifies important gene networks underlying growth and susceptibility to the weevil in spruce. In particular, we found transcripts that strongly modify the trade-off between growth and defense, and allow identification of networks more central to the trade-off.

Key words: gene networks, genetic correlations, growth, herbivory, perennials, *Picea*

41 INTRODUCTION

42

43 Coevolved host defenses and herbivores have a long-term evolutionary history (Futuyma &
44 Agrawal, 2009). The resource allocation towards growth on one side, and towards defense
45 and/or reproduction on the other side, is governed by suites of genes and their metabolic
46 pathways. Trade-offs between life-history traits involve the hierarchical allocation of
47 resources to biochemical pathways (Worley *et al.*, 2003). Thus, major plant defense theories
48 surrounding a trade-off between growth and defense mainly center on carbon/nutrient balance
49 and growth differentiation balance and the associated allocation costs for defense at the
50 expense of growth and reproduction (Bryant *et al.*, 1983; Herms & Mattson, 1992). It is well
51 known that certain signaling pathways usually required for developmental processes
52 (reproductive development, *e.g.*, Howe & Jander, 2008; Steppuhn & Baldwin, 2008) can be
53 co-opted for biotic stress responses that directly influence the performance of the pest or
54 contribute indirect defense responses to attract predators or herbivore parasitoids (Thaler *et*
55 *al.*, 2002). This provides the plant with inducible defenses against herbivores or pathogens
56 that are evolutionary advantageous (Steppuhn & Baldwin, 2008). Moreover, when plant
57 growth is decreased by drought for example, then, more carbon becomes available for the
58 production of secondary compounds. Thus, drought stress was shown to increase oleoresin
59 concentration in conifers' woody tissue with the consequence of higher resistance against
60 herbivory (Turtola *et al.*, 2003). Regarding the genetic underpinnings of a growth/defense
61 trade-off, genomic investigations can disentangle this myriad of interactions and demonstrate
62 evolutionary trade-offs between growth and defense. In this paper, we adopt partial
63 correlation analysis to help disentangle such trade-off interactions between growth and
64 defense in trees.

65 White spruce (*Picea glauca*) and Interior spruce (*P. glauca x engelmannii*) are the
66 economically most important forest tree species in British Columbia. However, both species
67 suffer from infestations by the spruce shoot weevil (*Pissodes strobi*), which consumes the
68 phloem of the apical leader and deforms the main stem (Alfaro *et al.*, 2004; vanAkker *et al.*,
69 2004; King *et al.*, 1997; He & Alfaro, 2000; Kiss & Yanchuk, 1991; Tomlin *et al.*, 1998).
70 Under the pressing need to understand the genetic control of resistance, studies with *Picea*
71 have illustrated host defenses to phloem feeding insects (Ralph *et al.*, 2006; Lippert *et al.*,
72 2007; Miller *et al.*, 2005; Byun-McKay *et al.*, 2006; McKay *et al.*, 2003). Ralph *et al.*
73 (2006) originally documented herbivore-induced transcriptome variation, reflecting either

reallocation of resources from primary processes to active defense, or the mobilization of the resources for host tolerance (Howe & Schaller, 2008). Anatomical and the associated chemical defenses in *Picea* bark have also been described (Franceschi *et al.*, 2005). The physical structures studied in most detail are the parenchyma cells, and the resin ducts that are all located in the secondary phloem and cambium; the traumatic resin canals are formed in the secondary xylem (Franceschi *et al.*, 2005).

In *Picea*, the genetic relationship of insect resistance with height growth is ambiguous (Kiss & Yanchuk, 1991; King *et al.*, 1997; Alfaro *et al.*, 1997; He & Alfaro, 2000; Lieutier *et al.*, 2003). In Sitka spruce, interestingly, genetic resistance was highest in families with only average growth rate (Alfaro *et al.*, 2008). Also, constitutive and induced defenses do not always follow sequentially: some resistant trees do not produce/rely on traumatic resinosis (a direct measure for resistance); however, some trees from susceptible families do defend with intensified resin flow to wounding (Alfaro, 1995).

Therefore, in the present study, our specific objective was to identify genes underlying the trade-off between growth rate and weevil resistance. In an experiment initiated by René Alfaro (Alfaro *et al.*, 2004), *Picea glauca x engelmannii* progenies were measured for pre-attack plant growth and post-attack damage with several morphological measures, which formed the basis of our hypotheses about correlations. We then estimated gene expression in bark tissue from terminal leaders using microarrays spotted with 13,980 ESTs and their annotated respective transcripts. For network inference, we used a new approach where, with respect to joint values of growth and weevil susceptibility (host defense) traits, we identified a subset of transcripts that exhibited either strong excessive (1) positive partial phenotypic correlations or (2) negative partial phenotypic correlations. That is to say, we chose ESTs that had strong indirect effects on both growth and susceptibility to the weevil, as either jointly positive or jointly negative. These subsets of transcripts were then subject to network analysis, but using entire correlations as the measure of similarity. Our results illustrate how partial correlation analysis of transcript levels can help reveal genes involved in the trade-off between growth and resistance.

MATERIALS AND METHODS

Interior spruce pedigree

The experimental population of Interior spruce (*Picea glauca* (Moench) Voss x *P. engelmannii* Parry ex Engelm.) at the Kalamalka Research Station in Vernon, British Columbia (BC), Canada, established in 1995, comprises of 42 full-sib families, each of size 75, from parents selected throughout the Prince George Seed Planning Zone in Northern BC (Alfaro *et al.*, 2004). On the basis of previous weevil attack, parents were classified as resistant (R) or susceptible (S), so that families could be grouped as deriving from two resistant parents (R*R: 16 families), one resistant and one susceptible parent (R*S: 20 families), or both susceptible parents (S*S: 6 families). These 42 families were scored for growth and resistance traits. The experimental setup and sampling details are as described in Porth *et al.* (2011).

Phenotypic assessments

Initial (early spring) height was recorded in years 1995-1999, terminal leader length was measured in 1999, and weevils were artificially applied in October 1999. After Alfaro *et al.*, (2004), attack rates in 2000 and 2001 were classified as (1) successful top kills, (2) attack but no death of leader, and (3) 'no attack'. The number of eggs laid (oviposition) was also visually recorded as five discrete classes of egg punctures: (1–25), (26–50), (51–75), (76–100), (101+); these were easily distinguished from feeding punctures (R. Alfaro, pers. comm.). The sums of weevil attacks and oviposition for 2000 and 2001 were also used as resistance traits. For the 1999 growing season, bark histology measures were taken for 10 trees per cross, with resin duct measurements taken on upper laterals closest to the leader (Alfaro *et al*., 2004). In the present study we retained only such resin canal characteristics where a positive linear relationship between the leader and the laterals from the same whorl had been shown (Alfaro *et al.*, 2004). The following abbreviations for labels and formulas related to bark histology measures were used that followed those provided in Alfaro *et al*., (2004): AREALRC [area of large resin canals (microns squared)], AREASRC [area of small resin canals (microns squared)], TAREARC [total area of resin canals, large plus small (microns squared)], LRC_BA [AREALRC/BARKAREA], SRC_BA [AREASRC/BARKAREA], TRC_BA [TAREARC/BARKAREA], NOLRC [number of large resin canals], NOSRC [number of small resin canals], TOTNORC [total number of resin

canals (large plus small)], SZ_IN [AREALRC/NOLRC], NMMS_IN [NOLRC/BAMM], NMMS_OUT [NOSRC/BAMM], NMMS_TOT [TOTNORC/BAMM], BTHK [bark thickness in mm], BARKAREA [total quadrant bark area (square microns)], and BAMM [bark area in mm squared].

Tissue collection, RNA preparation, microarray, and gene expression profiling

Weevil activity was also observed when we sampled in spring of 2006, and precise records of attacks on-site due to an elevated weevil population were available from 2000-2003. However, for this particular experiment, newly attacked shoots/tree leaders were intentionally excluded from sampling in order to be able to perform an experiment on constitutive levels of defense traits. Thus, we sampled at the right time point, when natural weevil activity occurred in the field. Bark and phloem tissue were collected in the mornings of May 16-18, 2006 and frozen in liquid nitrogen. As we were measuring gene expression, every effort was made to randomize and standardize the collections of tissue. Total RNA was isolated following (Kolosova *et al.*, 2004) and quantified via NanoDrop® ND-1000 spectrophotometer; RNA integrity was evaluated using Agilent 2100 Bioanalyzer. The 21,840 spruce ESTs microarray we used for gene expression profiling in this study and the microarray's quality control are described elsewhere (S. Ralph and co-workers, Gene Expression Omnibus database GEO: GPL5423). A total of 13,980 annotated spruce EST elements were retained for this study. Hybridizations and image acquisition were carried out as described in Ralph *et al.* (2006) and Porth *et al.*, (2011).

Microarray experimental design and processing of data

A subset of four R*S families, with wide segregation for weevil resistance, were chosen for the gene expression assay. The hybridizations profiled 188 individuals: 48, 36, 50, and 54 in crosses #26, #27, #29, and #32, respectively. After quantitation of the signal intensities in each array, the local background was subtracted for each sub-grid. Data were further normalized using the variance stabilizing normalization method implemented in R package 'vsn' (Huber *et al.*, 2002). All slides underwent simultaneous normalization to yield a similar overall expression level and variance for each channel independent of the array. A linear model that incorporated dye and block effects in a two-colour microarray design was used (Porth *et al.*, 2011). Signal intensities for the original and the normalized measurements were deposited in GEO under GSE22116.

168

169 *Partial correlation analyses*

170 Using family means, we estimated genetic correlations in the 42 *P. glauca x engelmannii*
171 crosses. We found: 1) genetic correlations between growth and susceptibility were negative
172 but not strong (r~-0.2, P<0.05), 2) genetic correlations between bark histology measures and
173 attack severity were negative and stronger (r~-0.5, P<0.05), and 3) genetic correlations
174 between histology and growth were all positive (Fig. S1). These are in accord with previous
175 studies (Kiss & Yeh 1987; Kiss & Yanchuk 1991; King *et al.*, 1997) and serve to frame our
176 hypothesis that growth and attack have a negative genetic correlation, but show an overall
177 positive phenotypic correlation (r=0.85, P<0.05).

178 To explore the gene networks underlying relations between growth and attack, we
179 used partial correlation analysis to examine how the correlation between two variables
180 (growth and attack) is influenced by a third variable, a gene expression locus (E). As we
181 assayed 13,980 ESTs, we sought to identify ESTs of major effect, and to identify these, most
182 obviously we can inspect bivariate relationships. Fig. 1 plots the observed amounts of growth
183 and attack across 1255 ESTs that had a significant association (P<0.01) of expression with
184 the two traits (growth was measured by leader length in yr 1999, and attacks were summed
185 over yrs 2000 and 2001; correlations were recomputed in 1,000 randomized datasets). The
186 phenotypic strength of association almost exactly matches the observed phenotype correlation
187 of 0.85 between growth and attack, but this by itself does not provide any further inferences,
188 especially about networks.

189 To this end, we employ the logic of partial correlation to find nodes that are more
190 central to the gene network underlying growth and defense. Let E be an expressed gene, X be
191 one trait (growth or susceptibility) and Y be a second trait (growth or susceptibility). The
192 following are the observed phenotypic correlations: r_{XY} between X and Y, r_{XE} between X and
193 E, and r_{YE} between Y and E. The partial correlation between X and Y, that is the correlation
194 after the confounding effect of E is removed, is

195 $$r_{XY.E} = \frac{r_{XY} - r_{XE} r_{YE}}{\sqrt{1 - r_{XE}^2}\sqrt{1 - r_{YE}^2}} \quad (1)$$

196 A significant partial correlation can be caused by a mutual effect of E on both X and Y; this
197 can occur when E is at the more central node to X and Y, relative to other possible nodes. If
198 r_{XE} and r_{YE} are either both positive or negative, the partial correlation will be reduced,
199 otherwise the partial correlation will be increased. Regardless of the direction of change, the

amount of change is proportional to the relative importance of *E* in the network of *X* and *Y*. A measure of the relative change due to the introduction of *E* is

$$d_{XY.E} \equiv (r_{XY} - r_{XY.E})/r_{XY} \quad (2)$$

Greater values of $d_{XY.E}$ occur when *E* explains a greater fraction of the correlation between *X* and *Y*, or generally when *E* is in a tighter network with *X* and *Y*.

We found the more direct connections within a network by systematically evaluating Equation (2) for all pairs of quantitative traits *X* and *Y*, for each EST *E*. Values of $d_{XY.E}$ greater than a significance threshold are retained in the network; executing this over all possible edges results in a network of putative direct interactions. In constructing the final network, we used total correlations as opposed to the partial correlation quantities in Eq. 2, which only serve to identify ESTs to retain in the network.

A partial correlation threshold network (PCTN) can also be established with rigorous application of a threshold (Kenett *et al*., 2010), but we simply identified two groups. One group of 21 had all positive $d_{XY.E}$ values and the second group of 166 had all negative $d_{XY.E}$ values; these groups are described in Notes S1. Significance was again determined by randomization and ESTs of extremely high probability were retained (see Notes S1). One can imagine each group is the average of several tips in Fig. 1 (there are $n(n-1)/2$ tips for *n* traits). We note that, in principle, groups of ESTs with all ++ or – *X* vs *Y* effects, and with all +- and -+ *X* vs *Y* effects, could be grouped as co-variates in a multivariate partial correlation analysis.

220 RESULTS

221

222 *The genetic basis of susceptibility to weevil attack/growth trait correlations (genetic*
223 *pleiotropy) in P. glauca x engelmannii*

224 Total correlations in the two-by-two factorial R*S spruce progeny are given in Table 1. We
225 identified numerous significant positive and negative correlations between gene expression
226 levels for individual ESTs and the phenotypic traits (Table S1). In addition, *p*-values were
227 computed for each gene-by-growth effect, and then *q*-values were calculated to adjust for
228 false discovery rate (Storey & Tibshirani, 2003). The effect estimate for each gene was also
229 obtained representing the change in its expression per unit change in growth rate. This
230 identified 867 genes at $q<0.1$, Table S2.

231 We also determined the partial correlations by using the ESTs as independent
232 variables and estimated the relative differences between the total and the partial correlations
233 (Table 2). These pairwise comparisons (*i.e.* the pairwise growth-resistance trait comparisons
234 representing the partial correlation in either direction between growth and susceptibility to
235 weevil attacks) showed large transcriptome responses in terms of the partial correlations
236 representative of the pairwise comparisons between traits (Fig. S2) along with the different
237 effects (*i.e.* the negative and the positive directions, respectively) of the components of these
238 correlations between susceptibility to weevil attacks and growth (Notes S1 for details). Thus,
239 we could uncover all possible combinations related to our initial hypothesis testing regarding
240 the directionality of the genetic correlations to be tested with individual traits (Fig. 1).

241 We estimated the correlations' upward bounds involving the top 100 genes in the
242 positive and the negative correlations between the susceptibility to weevil attacks and growth,
243 respectively (Table 2). This provided a clear picture regarding a significant increase (top 100-
244) of the partial correlations coefficient compared to the phenotypic (total) correlations which
245 had shown a positive trend for growth and weevil attack (Table 2). Therefore, the results
246 relating to the partial correlations also indicated that we could uncover the genetic effect of
247 the transcriptome attributed to the negative genetic correlations between weevil attack and
248 height growth in spruce (see above). Still, we found transcripts that could enhance growth but
249 could also have a negative impact on resistance or vice versa (positive correlations), Table 2.

250

251 *Identification of candidate networks using partial correlations*

The Supplement material (Notes S1) gives the relative partial correlations (Eq. 2) for individual ESTs and each of the six pairwise combinations of the four traits. These four traits were ldr_99 (leader length in 1999), Ht_5yrs (height at age 5) and atktot (attack damage, total), eggtot (egg number, total). There were many significant positive and negative partial correlations between gene expression levels for individual ESTs and the phenotypic traits. Hence, there was sufficient power to use partial correlation analysis to detect networks of interest. Thus, most importantly, on the basis of the partial correlation analysis and Eq. 2, we identified two distinct compact networks (P=0.004 for all pairwise comparisons) for the positive correlations (++ or -- effects) involving 21 transcripts (represented by Fig. 2A) and for the negative correlations involving 166 transcripts (corresponding to +- or -+ effects), respectively (represented by Fig. 2B), and in Fig. 1, respectively. The details of the genes' identities for these two networks are provided in Table S3 (corresponding to the network presented in Fig. 2A) and in Table S4 (Fig. 2B). The complete lists of genes used in four transcript files (of sizes 100, 166 and 21, respectively) are given in Notes S1 as well as in Tables S3-S6.

The genes at the centre of pleiotropy between tree growth and pest resistance in P. glauca x engelmannii

A putative PDR type ABC transporter *ATPDR12/PDR12 (PLEIOTROPIC DRUG RESISTANCE 12*), represented as element WS0269_K02 on the 21.8k spruce EST array, showed the strongest significance among all 13,980 tested transcripts in the correlations between gene expression, tree height and weevil attack phenotypes (P= 0.019), Table 3. Variation in *PDR1*2 steady-state gene expression at the population level represents an example of transcripts that were positively correlated with height but negatively correlated with weevil susceptibility traits (+- effects) in the two-by-two factorial crosses progeny widely segregating for resistance. A set of additional genes showed the same directionality in gene expression with growth and resistance variation for this large progeny of 188 individuals originating from 4 different crosses (Table S1).

However, among transcripts that correlated negatively (-- effects) with both growth and attack/oviposition (again tested by 1,000 permutations), there were spruce genes with the following functions: (a) dirigent proteins related to constitutive defenses (array elements WS0086_I04 and WS0104_A04 annotated as *PicsiDIR29* and *PicsiDIR35*, respectively (Ralph *et al.*, 2007; Porth *et al.*, 2011)), (b) peroxidases important in the reinforcement of

anatomical structures through lignification (WS01029_G23, WS01033_K22, and WS01017_F04 (Porth *et al.*, 2011)), (c) other cell wall modifying proteins such as xyloglucantransglycosylases (WS0264_P12, WS0041_D16), xyloglycosyltransferases (WS0072_C14, WS00918_N14), pectinesterase (WS0039_I15), (d) stress inducible peroxidase *PicabPIPRX* (WS01029_D16 (Porth *et al.*, 2011)), stress signaling pathway related AP2 domain transcription factor *TINY* (WS0102_C24) (Sun *et al.*, 2008), (e) flavonoid-based defense related *F3'H* (WS00931_D17 (Porth *et al.*, 2011)), and finally (f) the weevil-inducible terpene synthase (WS0078_K20 (Kolosova, 2010)). Three candidates that showed the negative correlation with growth, the chaperonin heat shock protein 60 WS00920_E06, the ethylene inducible universal stress protein family protein WS01027_A08, as well as the terpene synthase WS00929_B22 were found to be significantly downregulated in weevil-resistant spruce trees (Verne *et al.*, 2011).

Our results indicated that expression of these three genes negatively correlated with attack rates, and their expression was also negatively correlated with growth; therefore we assume a potential trade-off. Among all these genes mentioned above, only three were represented in the 21-member gene-gene interaction network (the two spruce peroxidases potentially implicated in lignification, WS01033_K22, and WS01017_F04, as well as the spruce AP2 domain transcription factor *TINY* WS0102_C24), Fig. 2A and Table S3.

304 DISCUSSION

305

306 Our work investigated the co-evolution of height growth vigour and pest resistance in spruce
307 and employed transcriptomics data to obtain insights into this relationship. We assayed for
308 gene expression levels in a sample of four families (188 individuals in total) segregating for
309 weevil resistance. Gene expression levels were interrogated for 13,980 annotated ESTs
310 spotted on a 21.8k member microarray. We then estimated the partial correlations of pairs of
311 growth/attack traits with gene expression levels, and identified ESTs of high correlation
312 likely at the centre of such phenotypic trait correlations among growth and attack traits.

313 In conifers, the generalized strategy for protection against bark boring pests are the
314 constitutive defenses that are localized within the periderm, the cortex, the secondary phloem
315 and xylem and are arranged in concentric, multiple layers (Franceschi *et al.*, 2005). Toxins,
316 antifeedants, defensive proteins and enzymes, and reservoirs of chemicals such as resins are
317 released from the bark upon attack. The spruce shoot weevil attacks the host tree at the shoot
318 apical leader of previous year's growth (Kiss & Yanchuk, 1991). The apical leader unites the
319 high fitness value for the tree's competitiveness with high probability for weevil attack;
320 consequently, this part of the tree receives priority in the allocation of defense metabolites.
321 The attractiveness of the prospective host to the herbivore is highly related to the host's
322 nutritional adequacy (*e.g*. content of sterols, lipids, fatty acids, amino acids, carbohydrates).
323 Since preformed defenses are established coordinately during the development of secondary
324 xylem in the apical shoot (Friedmann *et al.*, 2007), the tree's innate metabolism related to
325 growth and normal development very likely influences the establishment of these defenses
326 directly, thus forming the foundation for genetic pleiotropy. In the present study, we further
327 explored the evidence of pleiotropy between growth and constitutive resistance against the
328 herbivore in the host (Porth *et al.*, 2012) including a potential trade-offs.

329 We investigated the correlations between growth and resistance traits involving
330 transcript expression to identify genes that might be at the centre of potential trade-offs
331 between inherent growth rate and constitutive defenses. Transcriptomics can uncover
332 causality in true interactions between genes, if in the analysis of pairwise gene interactions all
333 other transcripts are also considered (partial correlation analysis, c.f. Johansson *et al.*, 2011).
334 Consequently, our correlation networks based on such analysis highlighted the more direct
335 associations among the myriad of all possible associations.

336

JA biosynthesis genes

Hormonal crosstalk is indispensable for regulating the growth-defense trade-offs and jasmonate (JA) signaling lies at the centre of networks that define defense strategies against herbivory (Huot *et al.*, 2014). The differential regulation of certain components/steps in the JA pathway generates distinct responses to different stimuli (reproductive development, growth or types of defenses that can be active defenses (Kazan & Manners, 2008)). Following the apparency theory, fast growing individuals are thought to be biased towards induced defenses (Steppuhn & Baldwin, 2008). The chemical compounds providing active (induced) protective defenses against herbivory are the terpenoids and some phenolic derivates (such as tannins (Bauce *et al.*, 2006)). In general, the induced defenses rather acquire signaling systems from developmental programs; and such defenses are expressed only when required. Hence, such types of defenses primarily allow resource investment for the plant's competitiveness through increased growth (and reproduction). For example, one study failed to connect defensive tannin production with a growth trade-off (Haering *et al.*, 2008), but see further below. In our study, the levels of JA defense signaling in fast growing (unattacked) progeny were lowered. Genes involved in the plastid located early JA biosynthetic steps (*LOX1*: WS01014_A24; *AOS CYP74A*: WS01016_F05 and *AOC*2 genes: WS00820_E17) all showed decreases in steady-state transcript abundance in fast growers (Table S2). We also retrieved these genes in the compact 166-member network involving the negative correlations between growth and attack (Fig. 2B). Also, the majority (c.70%) of the suite of the spruce genes involved in reproductive (male and female strobili) development, identified to be differentially expressed, exhibited increases in steady-state transcript abundance in fast growing (unattacked) progeny (Table S2).

Terpenoid-based defense

We identified two spruce genes with roles in terpenoid defense against herbivory in the compact 166-member network (Fig. 2B). First, we found *PDR12*, which is possibly involved both in constitutive as well as in induced defense. In *Arabidopsis*, *PDR12* shows a response to infection by necrotrophic fungal pathogens but its upregulated gene expression can also be artificially elicited by applications of methyljasmonate, salicylic acid and ethylene in growth media; its study revealed the first evidence of *active* transport of terpenoids in plant defenses (Campbell *et al.*, 2003; van den Brule & Smart, 2002). Second, we also identified a putative linalool oxygenase (*CYP76C1*) with a potential role in detoxification during plant-insect

interactions (Hofer *et al.*, 2014). Interestingly, expression levels of these two genes showed marked differences with growth rate, with *PDR12* increasing in steady-state transcript abundance in fast growers. Other spruce genes implicated in terpenoid metabolism including genes from the methylerythritol phosphate pathway, monoterpene (myrcene, pinene), sesquiterpene (farnesene), or tocopherol biosynthesis exclusively showed increases in steady-state transcript abundance in fast growers. Tocopherols promote stress tolerance by protecting against oxidative stress, while terpenoids have mostly active defensive functions. Interestingly, these genes were not identified in previous weevil feeding experiments (where a very limited number of tested genotypes was included), thus they were also not functionally characterized previously (Ralph *et al.*, 2006; Keeling *et al.*, 2011). Possibly, the phenotypic expression of tolerance and well-established chemical defenses towards herbivory is mutually exclusive.

Flavonoid-based defense

Several flavonoid pathway-related genes were part of the 166-member network (Fig. 2B; Table S4). Functions were related to the biosynthesis of anthocyanins/tannins (protective function against herbivorous insect damage and/or associated fungi (Bauce *et al.*, 2006; Hammerbacher *et al.*, 2014) and biosynthesis of lignans (via phenylcoumaran benzylic ether reductases, *PBR* (Macrae & Towers, 1984)). And while *PBR PicglPPR0*5 was associated with attack rates, there was also evidence that this gene was tightly regulated by a secondary growth related transcription factor (*myb20*), and showed decreases in steady-state transcript abundance with increased growth rates (Porth *et al.*, 2011), interestingly, with a positive effect on genetic resistance (Table S4). *CYP750* members were related to weevil resistance (Porth *et al.*, 2011) but also to growth traits with differentially correlated gene expression. Although such conifer-specific P450 members are supposedly involved in a wide range of derivatization reactions in phenylpropanoid metabolism, their exact functions need yet to be ascertained. Dedicated tannin related and downstream regulated genes showed either no or a positive relationship with growth rate (Table S2) suggesting similar findings as a previous study that failed to relate tannin production to a trade-off (Haering *et al.*, 2008). This pattern was opposite to the above mentioned early flavonoid pathway gene *CYP7*5/*F3'H* expression implicated in a potential trade-off. Possible reasons for such trade-off are the cross-talk between the lignin and the flavonoid pathways (metabolic plasticity (Mouradov & Spangenberg, 2014)) and the additional role flavonoids play in plant development (auxin

transport: Taylor & Grotewold, 2005). In addition, other studies exist, which showed trade-offs based on phenylpropanoids and condensed tannins, particularly under stress conditions that would change plants' allocation choices (*e.g.* pine trees: Sampedro *et al.*, 2011; Salicaceous trees: McKown *et al.*, 2014). Because of such interrelations, further investigations are warranted that also test for plants' growth-defense relationships under different stress conditions, including such comparisons for constitutive vs induced chemical defenses (see also below).

Reproductive Development

We identified spruce MADS-box genes that are candidates for reproductive maturity or reproductive meristem identity in conifers [annotated as *AGL20* (S*uppressor of* O*verexpression of* C*onstans 1*), *SOC1-like* gene *AGL42*, and *AGL2* (*SEPALLATA1*) based on sequence homology with *Arabidopsis* genes] (Katahata *et al.*, 2014; Uddenberg *et al.*, 2013; Melzer *et al.*, 2010) which are paralogs of flowering genes in *Arabidopsis*. As there are no *SEP1* genes in gymnosperms, the identified *P. glauca* gene WS00823_F11 is likely the ortholog of *P. abies DEFICIENS AGAMOUS-LIKE1* (PgMADS10_DAL1, with proposed function in regulating the transition from juvenile to adult phase in *Picea* (Carlsbecker *et al.*, 2004)), and which is located within the *SEP1* sister clade more closely related to *AGL6*. Likewise, *SOC1* and *SOC1-like* annotated *P. glauca* genes, WS0056_A03 and WS00922_C06, respectively, are homologs of *P. abies DAL3* (PaMADS7_DAL3 (Melzer *et al.*, 2010)). Functional analysis of *CjMADS14*, the *Cryptomeria japonica* ortholog of *P. abies DAL1*, and *CjMADS15*, the *C. japonica* ortholog of *P. abies DAL3*, indicated that expression of *DAL1* is specific to the reproductive organs (and its function is related to suppressing reproductive repressors), while expression of *DAL3* is more ubiquitous but included male and female strobili (Katahata *et al.*, 2014; Niu *et al.*, 2016).

In both the negative and the positive correlation networks, we found the *P. glauca DAL3* homolog WS00922_C06, as well as the *P. glauca DAL1* gene and the other *P. glauca DAL3* homolog, WS0056_A03. Expression of the *P. glauca DAL3* homolog WS0056_A03 showed significant increases in steady-state transcript abundance in fast growing spruce individuals ($q<0.05$) (conversely to *DAL1* whose expression pattern was unaffected in either direction by growth) and its expression was associated with the identified growth-resistance trade-off, while the second *DAL3* homolog was significantly down-regulated in fast growers ($q<0.05$) and part of the negative correlation between growth and attack (Fig. 2B). These

results are indicative of opposing functions of the two *DAL3* homologs and a striking example of subfunctionalization in the extensively expanded and duplicated clade of TM3-like genes in *Pinaceae* (Gramzow *et al.*, 2014). Interestingly, *DAL3* is closely related to the *P. abies acrocona* (*acr42124_1*/*DAL19*) gene, which promotes early cone-setting (Uddenberg *et al.*, 2013), and to which WS0056_A03 would be the closest of the two white spruce homologs. It is important to mention here that similar to the *P. abies acrocona* phenotype (Uddenberg *et al.*, 2013), an enhanced WS0056_A03 expression is also negatively correlated with expression of genes involved in cell wall modification, cell signaling, and plant stress response (Fig. 2A and Table S3). Such gene expression pattern was identified as a trade-off between enhanced growth and weevil resistance.

Different genomic introgression patterns in Interior spruce

We would like to highlight the putatively different genomic introgression patterns among the studied *P. glauca* x *P. engelmannii* hybrid progeny as one possibility to explain the differences in the relationship between growth and resistance among individuals, as, for example, it is known that pure *P. glauca* grows at different rates than pure *P. engelmannii* or their respective hybrids, and this relationship also differs for different life-history stages; while *P. glauca* initially grows more slowly, it outpaces *P. engelmannii* after tree age 10 (De La Torre *et al.*, 2014). Current tree breeding programs select towards more vigorous trees thus higher *P. glauca* ancestry, and concurrently restrict the downward displacement of Engelmann spruce seedlots due to increased weevil susceptibility. We point out that there may be differences in selection pressure on the pure species, as low elevation populations are much more resistant to weevils than their high elevation counterparts (which mainly consist of *P. engelmannii*), where weevils cannot thrive. However, there is currently no scientific evidence for significant differences in weevil resistance between the pure species (B. Jaquish, personal obs.).

Conclusions and prospects

Research on the relationships between plant growth and defenses against herbivory is timely, especially, when physiological with molecular approaches are combined to predict trade-offs (Züst & Agraval, 2017). Thus, for functional genomics studies of host defense mechanisms deployed against herbivores, it is important to consider inherent growth characteristics of the host. This research has not received enough attention in forest trees, although tree breeding

strategies seek to optimize the growth-defense balance, and ideally maximize for both growth and defense. Such obtained knowledge is particularly important for conifer trees species, which are widely planted and are already undergoing advanced generation breeding programs, in contrast to deciduous tree species, such as poplars.

Here, we used partial correlation analysis to identify the key genes and network nodes based on gene expression associated with phenotypic and genetic variability in the context of tree resistance to a major pest. Overall, our study did not show a trade-off between high growth rates and defenses. Nevertheless, important environmental components could influence the relationship between growth and pest resistance. Previously assessed phenotypic correlations that were found to be largely positive between shoot growth and susceptibility against weevil attacks would indeed hint at underlying environmental components. To further untangle the gene regulatory networks underlying the conifer tree's life history strategy, we are now investigating *ABCG40*/*PDR12* gene expression after wounding, under the alternative conditions of (1) drought stress, and (2) no drought stress imposed. It has been postulated that drought stressed conifers can rely more on constitutive than on induced defenses. If this candidate gene is involved with the trade-off, we can further characterize the role of *ABCG40* in relation to stress, and perhaps, separate the role of constitutive versus induced responses. We can also immediately apply such knowledge to breeding efforts against the stem boring pest, particularly for known local stress conditions, such as drought.

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AUTHORS' CONTRIBUTIONS

I.P., B.J. and K.R. performed experiments, conducted fieldwork, and analyzed data. R.W. provided statistical assistance. I.P. and K.R. drafted the manuscript. K.R. and I.P. planned and designed the research.

503 REFERENCES

504

505 **Alfaro RI. 1995.** An induced defense reaction in white spruce to attack by the white-pine weevil,
506 Pissodes strobi. *Canadian Journal of Forest Research-Revue Canadienne De Recherche*
507 *Forestiere* **25**(10): 1725-1730.
508 **Alfaro RI, He FL, Tomlin E, Kiss G. 1997.** White spruce resistance to white pine weevil
509 related to bark resin canal density. *Canadian Journal of Botany-Revue Canadienne De*
510 *Botanique* **75**(4): 568-573.
511 **Alfaro RI, VanAkker L, Jaquish B, King J. 2004.** Weevil resistance of progeny derived from
512 putatively resistant and susceptible interior spruce parents. *Forest Ecology and*
513 *Management* **202**(1-3): 369-377.
514 **Alfaro RI, King JN, Brown RG, Buddingh SM. 2008.** Screening of Sitka spruce genotypes for
515 resistance to the white pine weevil using artificial infestations. *Forest Ecology and*
516 *Management* **255**(5-6): 1749-1758.
517 **Bauce E, Kumbasli M, Van Frankenhuyzen K, Carisey N. 2006.** Interactions among white
518 spruce tannins, Bacillus thuringiensis subsp kurstaki, and spruce budworm (Lepidoptera :
519 Tortricidae), on larval survival, growth, and development. *Journal of Economic*
520 *Entomology* **99**(6): 2038-2047.
521 **Bryant JP, Chapin FS III, Klein DR. 1983.** Carbon/nutrient balance of boreal plants in relation
522 to vertebrate herbivory. *Oikos* **40**(3):357–368.
523 **Byun-McKay A, Godard KA, Toudefallah M, Martin DM, Alfaro R, King J, Bohlmann J,**
524 **Plant AL. 2006.** Wound-induced terpene synthase gene expression in sitka spruce that
525 exhibit resistance or susceptibility to attack by the white pine weevil. *Plant Physiology*
526 **140**(3): 1009-1021.
527 **Campbell EJ, Schenk PM, Kazan K, Penninckx I, Anderson JP, Maclean DJ, Cammue**
528 **BPA, Ebert PR, Manners JM. 2003.** Pathogen-responsive expression of a putative
529 ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is
530 regulated by multiple defense signaling pathways in Arabidopsis. *Plant Physiology*
531 **133**(3): 1272-1284.
532 **Carlsbecker A, Tandre K, Johanson U, Englund M, Engstrom P. 2004.** The MADS-box
533 gene DAL1 is a potential mediator of the juvenile-to-adult transition in Norway spruce
534 (Picea abies). *Plant Journal* **40**(4): 546-557.
535 **De La Torre AR, Wang T, Jaquish B, Aitken SN. 2014.** Adaptation and exogenous selection
536 in a Picea glauca x Picea engelmannii hybrid zone: implications for forest management
537 under climate change. *New Phytologist* **201**(2): 687-699.
538 **Franceschi VR, Krokene P, Christiansen E, Krekling T. 2005.** Anatomical and chemical
539 defenses of conifer bark against bark beetles and other pests. *New Phytologist* **167**(2):
540 353-375.
541 **Friedmann M, Ralph SG, Aeschliman D, Zhuang J, Ritland K, Ellis BE, Bohlmann J,**
542 **Douglas CJ. 2007.** Microarray gene expression profiling of developmental transitions in
543 Sitka spruce (Picea sitchensis) apical shoots. *Journal of Experimental Botany* **58**(3): 593-
544 614.
545 **Futuyma DJ, Agrawal AA. 2009.** Macroevolution and the biological diversity of plants and
546 herbivores. *Proceedings of the National Academy of Sciences of the United States of*
547 *America* **106**(43): 18054-18061.

Gramzow L, Weilandt L, Theissen G. 2014. MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. *Annals of botany* **114**(7): 1407-1429.

Haering DA, Huber MJ, Suter D, Edwards PJ, Luescher A. 2008. Plant Enemy-derived Elicitors Increase the Foliar Tannin Concentration of Onobrychis viciifolia Without a Trade-off to Growth. *Annals of Botany* **102**(6): 979-987.

Hammerbacher A, Paetz C, Wright LP, Fischer TC, Bohlmann J, Davis AJ, Fenning TM, Gershenzon J, Schmidt A. 2014. Flavan-3-ols in Norway Spruce: Biosynthesis, Accumulation, and Function in Response to Attack by the Bark Beetle-Associated Fungus Ceratocystis polonica. *Plant Physiology* **164**(4): 2107-2122.

He FL, Alfaro RI. 2000. White pine weevil attack on white spruce: A survival time analysis. *Ecological Applications* **10**(1): 225-232.

Herms DA, Mattson WJ. 1992. The dilemma of plants: To grow or defend. *Quarterly Review of Biology* **67**(3): 283-335.**Hofer R, Boachon B, Renault H, Gavira C, Miesch L, Iglesias J, Ginglinger J-F, Allouche L, Miesch M, Grec S, Larbat R, Werck-Reichhart D. 2014.** Dual Function of the Cytochrome P450 CYP76 Family from Arabidopsis thaliana in the Metabolism of Monoterpenols and Phenylurea Herbicides. *Plant physiology* **166**(3): 1149-1161.

Howe GA, Jander G 2008. Plant immunity to insect herbivores. *Annual Review of Plant Biology*. Palo Alto: Annual Reviews, 41-66.

Howe GA, Schaller A 2008. Direct Defences in Plants and Their Induction by Wounding and Insect Herbivores. In: Schaller A ed. *Induced Plant Resistance to Herbivory*: Springer, 7-29.

Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. 2002. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics* **18**: S96-S104.

Huot B, Yao J, Montgomery BL, He SY. 2014. Growth-Defense Trade-offs in Plants: A Balancing Act to Optimize Fitness. *Molecular Plant* **7**(8): 1267-1287.

Johansson A, Loset M, Mundal SB, Johnson MP, Freed KA, Fenstad MH, Moses EK, Austgulen R, Blangero J. 2011. Partial correlation network analyses to detect altered gene interactions in human disease: using preeclampsia as a model. *Human Genetics* **129**(1): 25-34.

Katahata S-I, Futamura N, Igasaki T, Shinohara K. 2014. Functional analysis of SOC1-like and AGL6-like MADS-box genes of the gymnosperm Cryptomeria japonica. *Tree Genetics & Genomes* **10**(2): 317-327.

Kazan K, Manners JM. 2008. Jasmonate signaling: Toward an integrated view. *Plant Physiology* **146**(4): 1459-1468.

Keeling CI, Weisshaar S, Ralph SG, Jancsik S, Hamberger B, Dullat HK, Bohlmann J. 2011. Transcriptome mining, functional characterization, and phylogeny of a large terpene synthase gene family in spruce (Picea spp.). *BMC Plant Biology* **11**:43.

Kenett DY, Tumminello M, Madi A, Gur-Gershgoren G, Mantegna RN, Ben-Jacob E. 2010. Dominating Clasp of the Financial Sector Revealed by Partial Correlation Analysis of the Stock Market. *PLoS ONE* **5**(12): e15032.

King JN, Yanchuk AD, Kiss GK, Alfaro RI. 1997. Genetic and phenotypic relationships between weevil (Pissodes strobi) resistance and height growth in spruce populations of

British Columbia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **27**(5): 732-739.

Kiss G, Yeh FC. 1987. Heritability estimates for height for young interior spruce in British Columbia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **18**:158–162.

Kiss GK, Yanchuk AD. 1991. Preliminary evaluation of genetic-variation of weevil resistance in interior spruce in British-Columbia. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **21**(2): 230-234.

Kolosova N, Miller B, Ralph S, Ellis BE, Douglas C, Ritland K, Bohlmann J. 2004. Isolation of high-quality RNA from gymnosperm and angiosperm trees. *Biotechniques* **36**(5): 821-824.

Kolosova N. 2010. *Transcriptome analysis of conifer defense against bark beetle-associated blue-stain fungi and white pine weevil.* University of British Columbia Vancouver. 223 pages.

Lieutier F, Brignolas F, Sauvard D, Yart A, Galet C, Brunet M, van de Sype H. 2003. Intra- and inter-provenance variability in phloem phenols of Picea abies and relationship to a bark beetle-associated fungus. *Tree Physiology* **23**(4): 247-256.

Lippert D, Chowrira S, Ralph SG, Zhuang J, Aeschliman D, Ritland C, Ritland K, Bohlmann J. 2007. Conifer defense against insects: Proteome analysis of Sitka spruce (Picea sitchensis) bark induced by mechanical wounding or feeding by white pine weevils (Pissodes strobi). *Proteomics* **7**(2): 248-270.

Macrae WD, Towers GHN. 1984. Biological-activities of lignans. *Phytochemistry* **23**(6): 1207-1220.

McKay SAB, Hunter WL, Godard KA, Wang SX, Martin DM, Bohlmann J, Plant AL. 2003. Insect attack and wounding induce traumatic resin duct development and gene expression of (-)-pinene synthase in Sitka spruce. *Plant Physiology* **133**(1): 368-378.

McKown AD, Guy RD, Quamme L, Klápště J, La Mantia J, Constabel CP, El-Kassaby YA, Hamelin RC, Zifkin M, Azam MS. 2014. Association genetics, geography and ecophysiology link stomatal patterning in Populus trichocarpa with carbon gain and disease resistance trade-offs. *Molecular Ecology* **23**(23): 5771–5790.

Melzer R, Wang Y-Q, Theissen G. 2010. The naked and the dead: The ABCs of gymnosperm reproduction and the origin of the angiosperm flower. *Seminars in Cell & Developmental Biology* **21**(1): 118-128.

Miller B, Madilao LL, Ralph S, Bohlmann J. 2005. Insect-induced conifer defense. White pine weevil and methyl jasmonate induce traumatic resinosis, de novo formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. *Plant Physiology* **137**(1): 369-382.

Mouradov A, Spangenberg G. 2014. Flavonoids: A Metabolic Network Mediating Plants Adaptation to Their Real Estate. *Frontiers in Plant Science* **5**: 620.

Niu S, Yuan H, Sun X, Porth I, Li Y, El-Kassaby YA, Li W. 2016. A transcriptomics investigation into pine reproductive organ development. *New Phytologist* **209**(3): 1278-1289.

Porth I, Hamberger B, White R, Ritland K. 2011. Defense mechanisms against herbivory in Picea: sequence evolution and expression regulation of gene family members in the phenylpropanoid pathway. *BMC Genomics* **12**(1): 608.

Porth I, White R, Jaquish B, Alfaro R, Ritland C, Ritland K. 2012. Genetical Genomics Identifies the Genetic Architecture for Growth and Weevil Resistance in Spruce. *PloS One* **7**(9): e44397.

Ralph SG, Yueh H, Friedmann M, Aeschliman D, Zeznik JA, Nelson CC, Butterfield YSN, Kirkpatrick R, Liu J, Jones SJM, Marra MA, Douglas CJ, Ritland K, Bohlmann J. 2006. Conifer defence against insects: microarray gene expression profiling of Sitka spruce (Picea sitchensis) induced by mechanical wounding or feeding by spruce budworms (Choristoneura occidentalis) or white pine weevils (Pissodes strobi) reveals large-scale changes of the host transcriptome. *Plant Cell and Environment* **29**(8): 1545-1570.

Ralph SG, Jancsik S, Bohlmann J. 2007. Dirigent proteins in conifer defense II: Extended gene discovery, phylogeny, and constitutive and stress-induced gene expression in spruce (Picea spp.). *Phytochemistry* **68**(14): 1975-1991.

Sampedro L, Moreira X, Zas R. 2011. Costs of constitutive and herbivore-induced chemical defenses in pine trees emerge only under low resources availability. *Journal of Ecology* **99**(3): 818–827

Steppuhn A, Baldwin I 2008. Induced defenses and the cost-benefit paradigm. In: Schaller A ed. *Induced Plant Resistance to Herbivory*: Springer, pp. 61-83.

Storey JD, Tibshirani R. 2003. Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America* **100**(16): 9440-9445.

Sun S, Yu J-P, Chen F, Zhao T-J, Fang X-H, Li Y-Q, Sui S-F. 2008. TINY, a dehydration-responsive element (DRE)-binding protein-like transcription factor connecting the DRE- and ethylene-responsive element-mediated signaling pathways in Arabidopsis. *Journal of Biological Chemistry* **283**(10): 6261-6271.

Taylor LP, Grotewold E. 2005. Flavonoids as developmental regulators. *Current Opinion in Plant Biology* **8**(3): 317-323.

Thaler JS, Farag MA, Pare PW, Dicke M. 2002. Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. *Ecology Letters* **5**(6): 764-774.

Tomlin ES, Alfaro RI, Borden JH, He FL. 1998. Histological response of resistant and susceptible white spruce to simulated white pine weevil damage. *Tree Physiology* **18**(1): 21-28.

Turtola S, Manninen AM, Rikala R, Kainulainen P. 2003. Drought Stress Alters the Concentration of Wood Terpenoids in Scots Pine and Norway Spruce Seedlings. *Journal of Chemical Ecology* **29**(9): 1981-1995.

Uddenberg D, Reimegard J, Clapham D, Almqvist C, von Arnold S, Emanuelsson O, Sundstrom JF. 2013. Early Cone Setting in Picea abies acrocona Is Associated with Increased Transcriptional Activity of a MADS Box Transcription Factor. *Plant Physiology* **161**(2): 813-823.

van den Brule S, Smart CC. 2002. The plant PDR family of ABC transporters. *Planta* **216**(1): 95-106.

vanAkker L, Alfaro RI, Brockley R. 2004. Effects of fertilization on resin canal defences and incidence of Pissodes strobi attack in interior spruce. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere* **34**(4): 855-862.

Verne S, Jaquish B, White R, Ritland C, Ritland K. 2011. Global Transcriptome Analysis of Constitutive Resistance to the White Pine Weevil in Spruce. *Genome Biology and Evolution* **3**: 851-867.

684 **Worley AC, Houle D, Barrett SCH. 2003.** Consequences of hierarchical allocation for the
685 evolution of life-history traits. *American Naturalist* **161**(1): 153-167.
686 **Züst T, Agrawal AA. 2017.** Trade-offs between plant growth and defense against insect
687 herbivory: an emerging mechanistic synthesis. *Annual Reviews of Plant Biology* **68**: 513-
688 534.
689

690 SUPPORTING INFORMATION

691

692 **Figure S1**: Correlation structure for significant genetic correlations among 18 individual host
693 tree characteristics.
694 **Figure S2**: Transcriptome changes for the two-by-two factorial R*S spruce progeny in terms of
695 partial correlations.
696 **Table S1**: Full list of correlation results of gene expression for the two-by-two factorial R*S
697 spruce progeny and for the 10 quantitative traits.
698 **Table S2**: Effect of inherent growth rate variation on global transcriptome changes for the two-
699 by-two factorial R*S spruce progeny.
700 **Table S3**: Gene id's of the 21-member compact network (P=0.004).
701 **Table S4**: Gene id's of the 166-member compact network (P=0.004).
702 **Table S5**: Top100+ transcripts, among all four pairwise growth-resistance trait comparisons.
703 **Table S6**: Top100- transcripts, among all four pairwise growth-resistance trait comparisons.
704 **Notes S1**: Partial correlations for the two-by-two factorial R*S spruce progeny.
705 **Notes S2**: Correlation network 21+, full results.
706 **Notes S3**: Correlation network 166-, full results.
707

FIGURE LEGENDS

Figure 1: The joint distribution of gene expression in relation to growth (leader length in yr 1999) and susceptibility (number of attacks in yrs 2000 and 2001).

Caption **Figure 1**: Only ESTs that showed associations with a permutation probably of 0.01 or lower are shown. The two ends, which have unshaded circles, illustrate the two groups (*i.e.* the upward bounds in the positive or negative direction of correlations, respectively) that could be subject to separate partial correlation analysis (as demonstrated in the present study).

Figure 2: Trade-off vs No trade-off scenarios testing between spruce tree growth and host's weevil pest resistance for the two-by-two factorial R*S spruce progeny uncovered by correlating the expression of 13,980 (annotated) spruce genes in bark tissue of the apical shoot with host tree height growth and host resistance towards the weevil phenotypes.

Caption **Figure 2**:

In both networks, correlation coefficient cutoffs of 0.197 were employed (Notes S2 and S3 show entire results); the social networks were visualized using Pajek (vlado.fmf.uni-lj.si/pub/networks/pajek/) employing the Fruchterman-Reingold approach; dashed lines represent *negative* correlations, solid lines represent *positive* correlations. **A**, Correlation network representative of the *positive* genetic correlations between host tree growth and pest attack among individual traits involving height at age 5 (yr 1999), apical leader length in yr 1999 (green vertices), total attacks and a total number of egg plugs (yrs 2000-2001) (red vertices) and 21 gene transcripts (P=0.004) (black vertices, except the spruce *acrocona* ortholog is shown in magenta). Spruce element annotations are provided in Table S3. **B**, Correlation network representative of the *negative* genetic correlations between host tree growth and pest attack among individual traits involving height at age 5 (yr 1999), apical leader length in yr 1999 (green vertices), total attacks and a total number of egg plugs (yrs 2000-2001) (red vertices) and 166 gene transcripts (P=0.004) (black vertices, except the spruce *PDR12* ortholog is shown in blue). Spruce element annotations are provided in Table S4.

735 TABLES

736

737 **Table 1:** Number of significant correlations (P <0.05; 1,000 randomizations) between gene
738 expression levels testing 13,980 spruce genes and 10 phenotypic traits related to tree height and
739 weevil resistance, respectively, for the two-by-two factorial R*S spruce progeny.

trait	positive	negative	both	proportion positive
ldr_99	1,827	1,553	3,380	0.540
init_Ht	844	949	1,793	0.471
Ht_3yrs	925	719	1,644	0.563
Ht_5yrs	2,118	1,968	4,086	0.518
atk00	933	1,021	1,954	0.477
atk01	521	630	1,151	0.453
atktot	996	1,116	2,112	0.472
egg00	640	907	1,547	0.414
egg01	474	494	968	0.490
eggtot	846	993	1,839	0.460

740 Caption **Table 1**:
741 Key: ldr99: apical leader length in yr 1999; Init_Ht: initial tree height in yr 1995; Ht_3yrs: tree
742 height in yr 1997; Ht_5yrs: tree height in yr 1999; atk00: attacks in yr 2000; atk01: attacks in yr
743 2001; atktot: total attacks summed over yrs 2000 and 2001; egg00: oviposition in yr 2000;
744 egg01: oviposition in yr 2001; eggtot: total oviposition summed over yrs 2000 and 2001.

745 **Table 2**: Partial correlations between growth and susceptibility for the two-by-two factorial R*S
746 spruce progeny.

Groups	Trait pairs					
	ldr_99 x Ht_5yrs	ldr_99 x atktot	ldr_99 x eggtot	Ht_5yrs x atktot	Ht_5yrs x eggtot	atktot x eggtot
All	0.858	0.287	0.339	0.265	0.297	0.815
Top100+	0.845	0.121	0.185	0.029	0.138	0.777
Top100-	0.821	0.448	0.570	0.435	0.514	0.792
Tight21+	0.813	0.118	0.229	0.075	0.168	0.785
Tight166-	0.743	0.075	0.211	0.215	0.128	0.568

747 Caption **Table 2**:
748 Key: ldr99: apical leader length in yr 1999; Ht_5yrs: tree height in yr 1999; atktot: total attacks
749 summed over yrs 2000 and 2001; eggtot: total oviposition summed over yrs 2000 and 2001, in
750 the set of four crosses, grouped by partial correlation analysis where only expression elements
751 with the highest effects on the partial correlation either in the positive direction (+) or negative
752 direction (-) are included in the groups, in groups of size 21, 100 and 166.

753 **Table 3**: Twenty-eight most significant transcripts from gene expression correlations for the two-by-two factorial R*S spruce progeny
754 with Ldr99, Ht99, atk00, atktot, egg00, eggtot phenotypes (*P*-value < 0.05 in bold; 1,000 permutations), sorted by overall significance
755 among all 13,980 tested transcripts (see Table S1).

Query ID	E-value	AGI #	Annotation	Ldr99	Ht99	atk00	atktot	egg00	eggtot	Overall *P*-value
WS0269_K02#‡	6.60E-154	AT1G15520	ATPDR12 (pleiotropic drug resistance 12)	**0.170**	**0.224**	-0.116	**-0.183**	**-0.142**	**-0.175**	0.0190
WS00915_B14*	2.20E-31	AT3G19380	U-boxdomain-containing protein	**-0.163**	**-0.193**	**-0.154**	**-0.186**	**-0.188**	**-0.221**	0.0196
WS0073_B15#‡	1.70E-19	AT3G02890	PHD finger protein-related	**0.136**	**0.196**	-0.108	**-0.197**	-0.108	**-0.158**	0.0253
WS01031_A08†	1.9E-73	AT5G43940	Alcohol dehydrogenase 2	**0.124**	**0.128**	**0.249**	**0.255**	**0.214**	**0.246**	0.0263
WS0094_H14‡	5.9E-70	AT3G10300	calcium-binding EF hand family protein	0.119	**0.175**	**-0.116**	**-0.175**	**-0.120**	**-0.179**	0.0277
WS01041_H21*†	2.50E-52	AT4G25835	AAA-type ATPase family protein	**-0.160**	**-0.211**	**-0.139**	**-0.183**	**-0.181**	**-0.208**	0.0298
WS0093_G14	2.7E-30	AT1G32450	proton-dependent oligopeptide transport family protein	-0.110	-0.111	**-0.160**	**-0.219**	**-0.223**	**-0.241**	0.0300
WS0093_H01*†	1.80E-52	AT3G07080	membrane	**-0.175**	**-0.231**	**-0.246**	**-0.244**	**-0.184**	**-0.183**	0.0329

			protein							
WS00824_D13#	1.10E-90	AT3G02750	protein phosphatase 2C family protein	**0.149**	**0.222**	0.118	**0.200**	0.072	**0.137**	0.0348
WS0261_D10	1.5E-89	AT4G28250	ATEXPB3 (Arabidopsis thaliana expansin B3)	0.113	**0.149**	**0.138**	**0.193**	**0.159**	**0.168**	0.0356
WS0101_H20	7.5E-28	AT2G44090	similar to unknown protein [A. thaliana]	-0.106	**-0.141**	**-0.174**	**-0.207**	**-0.132**	**-0.201**	0.0365
WS00920_D17*‡	2.60E-60	AT4G39230	isoflavone reductase, putative	**-0.235**	**-0.339**	0.082	**0.129**	**0.165**	**0.200**	0.0391
WS00933_P08	2.3E-24	AT5G22650	histone deacetylase 2B	**-0.130**	**-0.150**	**-0.224**	**-0.211**	**-0.147**	**-0.159**	0.0398
WS00812_N05	2.2E-30	AT3G02280	flavodoxin family protein	**0.129**	**0.144**	0.113	**0.183**	0.074	**0.137**	0.0402
WS00917_A11	9.8E-29	AT2G32060	40S ribosomal protein S12	**-0.222**	**-0.163**	**-0.152**	**-0.209**	**-0.203**	**-0.213**	0.0403
WS0039_H23	1.2E-30	AT1G50640	ethylene responsive element binding factor 3	**-0.128**	**-0.134**	**-0.177**	**-0.198**	**-0.190**	**-0.219**	0.0405
WS0091_H20†	4.30E-19	AT3G16640	translationally controlled tumor protein	**-0.212**	**-0.196**	**-0.173**	**-0.206**	**-0.136**	**-0.172**	0.0416

WS0102_O01	1.30E-56	AT4G31330	similar to unknown protein	**-0.121**	**-0.163**	**-0.194**	**-0.184**	**-0.193**	**-0.197**	0.0442
WS0076_N15#	2.50E-30	AT2G28080	glycosyltransferase family protein	**0.191**	**0.177**	**0.130**	**0.141**	**0.174**	**0.170**	0.0460
WS0024_M02	7.8E-178	AT5G63890	histidinol dehydrogenase	**0.155**	**0.121**	0.113	**0.153**	0.088	**0.157**	0.0465
WS0076_E13‡	5.40E-60	AT3G26070	plastid-lipid associated protein PAP / fibrillin family protein	**-0.175**	**-0.203**	0.089	**0.168**	**0.130**	**0.188**	0.0467
WS0264_J01	6.8E-92	AT5G51550	phosphate-responsive 1 family protein	-0.096	-0.116	**-0.134**	**-0.172**	**-0.138**	**-0.178**	0.0469
WS0072_P16‡	1.7E-49	AT2G02540	zinc finger homeodomain 4	**0.133**	**0.162**	-0.080	**-0.166**	**-0.156**	**-0.198**	0.0478
WS0262_A22	1.5E-34	AT5G56450	mitochondrial substrate carrier family protein	**0.144**	**0.137**	**0.153**	**0.218**	**0.127**	**0.188**	0.0486
WS00723_G19	1.20E-32	AT5G35170	adenylate kinase family protein	**0.186**	**0.173**	**0.236**	**0.213**	**0.171**	**0.175**	0.0488
WS0042_E16‡	3.9E-128	AT2G47470	PDI-LIKE 2-1, maternal effect embryo arrest 30, unfertilized embryo sac 5	0.115	0.115	**-0.171**	**-0.191**	**-0.161**	**-0.191**	0.0490
WS00917_K05#†	5.20E-07	AT4G30410	similar to unknown protein	**0.315**	**0.367**	**0.158**	**0.166**	**0.134**	**0.138**	0.0495

WS00716_G05‡	4.80E-14	AT3G13600	calmodulin-binding family protein	**-0.127**	**-0.159**	**0.123**	**0.175**	0.070	**0.141**	0.0497

Caption **Table 3**:

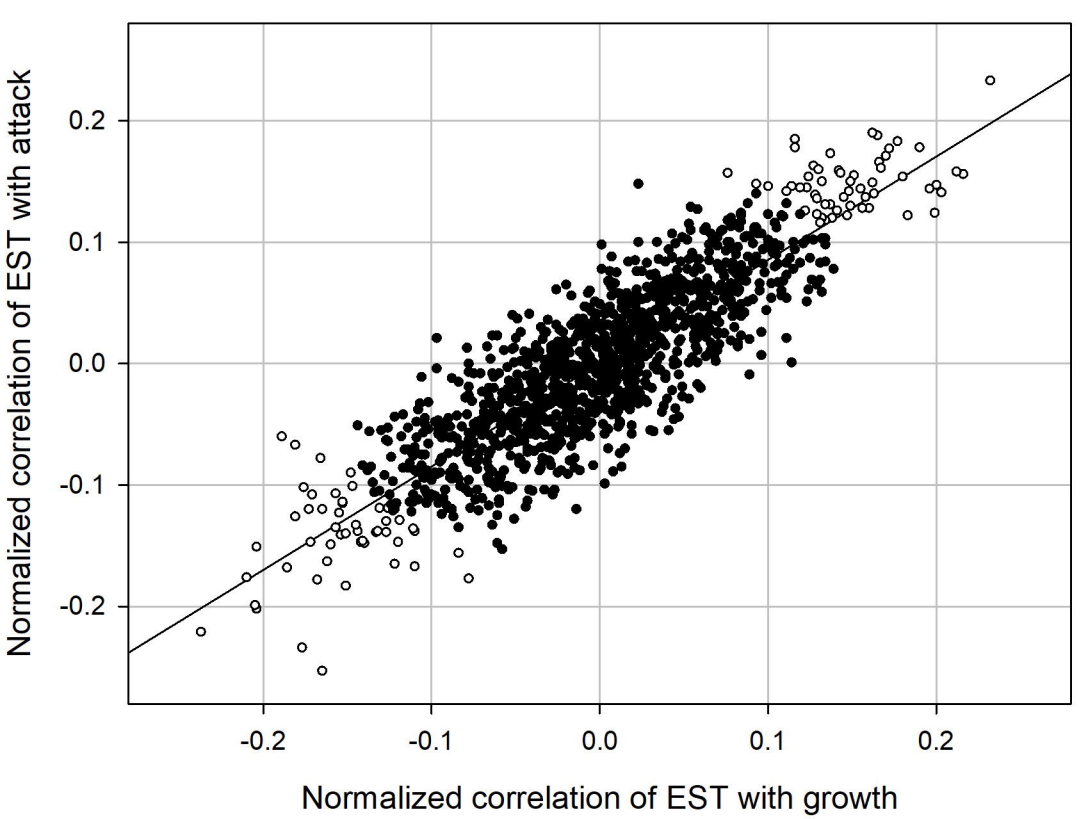
[#] Expression of these transcripts showed significant *increases* in steady-state transcript abundance in fast growing individuals ((Porth *et al.*, 2011) and Table S2).

* Expression of these transcripts showed significant *decreases* in steady-state transcript abundance in fast growing individuals ((Porth *et al.*, 2011) and Table S2).

[†]present in 21-member compact partial correlation network (P=0.004; Fig. 2A; Table S3), corresponding to trade-off scenario (Fig. 1).

[‡] present in 166-member compact partial correlation network (P=0.004), Fig. 2B (Table S4; see also Fig. 1).

Key: Ldr99: apical leader length in yr 1999; Ht99: height at age 5 (yr 1999), atk00: attacks in yr 2000; atk01: attacks in yr 2000, and atktot: total attacks; egg00: oviposition in yr 2000; egg01: oviposition in yr 2001, and eggtot: total number of egg plugs (yrs 2000-2001); sequence homology of the spruce cDNA to the respective Genbank entry (BLAST identity, AGI# for AT homolog) is supported by the expect value (E-value) of the hit.


Normalized correlation of EST with attack
0.2
0.1
0.0
-0.1
-0.2
-0.2
-0.1
0.0
0.1
0.2
Normalized correlation of EST with growth