Tissue-specific gene expression shows cynipid wasps repurpose host gene networks to create complex and novel parasite-specific organs on oaks

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Abstract

Every organism on Earth depends on interactions with other organisms to survive. In each of these interactions, an organism must utilize the limited toolbox of genes and proteins it possesses to successfully manipulate or cooperate with another species, but it can also coopt the genome machinery of its partner. Insect-induced plant galls are an extreme example of this, wherein an insect hijacks the plant genome to direct the initiation and development of galls comprising of plant tissue. However, the mechanism(s) behind insect-induced gall induction and development remain elusive. Here we demonstrate that cynipid wasp Dryocosmus quercuspalustris create a complex and novel parasite-specific organ from red oak tissue via massive changes in host gene expression. Our results show that the gall wasp is not merely modifying oak leaf tissue but creating a novel organ, resulting in extensive changes in gene expression between galled and ungalled tissue (differential expression in 28% of genes) and distinct gall tissue types (20% of genes). The outer gall tissue showed increases in various plant defense systems, which is consistent with its predicted functional role of protecting the wasp larva. The inner larval capsule shows suppression of large parts of the plant innate immune system and evidence for the wasp utilizing the plant's RNA interference mechanisms, which may be a potential mechanism of gall induction. We also find significant overlap between cynipid galls and agricultural gall pests, suggesting possible shared mechanisms for this complex species interaction even in disparate plants and insect galling guilds.

Introduction

Species interactions are a ubiquitous element of every ecosystem and food web on Earth (Agrawal et al., 2007; Bronstein, Alarcón, & Geber, 2006). These symbiotic interactions exist on a continuum from mutualistic to parasitic (Herre, Knowlton, Mueller, & Rehner, 1999; Johnson, Graham, & Smith, 1997). In each of these interactions, an organism is limited by the genes, proteins, and pathways it possesses to successfully manipulate or cooperate with another species, but it can also co-opt the genome machinery of its partner (e.g. virus replication, host manipulation by parasitoid wasps, plant flowering stimulated by bumblebees) (Martinson, Wheeler, Wright, Siebert, & Werren, 2014; Pashalidou, Lambert, Peybernes, Mescher, & De Moraes, 2020). Novel interactions often necessitate the creative use of existing pathways, especially in interactions between distantly related species (Martinson, Kelkar, Chang, & Werren, 2017; Wheat et al., 2007).

Tumor-like plant galls induced by insects are among the most fascinating structures found in nature. They are a product of novel and sophisticated species interactions that range from simple tissue swellings to complex and unique structures displaying pigments, forms, and defenses not normally produced by the the host plant (Ronquist & Liljeblad, 2001; Stone & Schönrogge, 2003). Insect-induced galls are an extreme example of an extended phenotype (Dawkins, 1999), where the gall is assembled and maintained with plant

genes and proteins, but the initiation, development, and morphology of galls are controlled by the insect by manipulating the meristem cells of the plant (Shorthouse & Rohfritsch, 1992). To create a successful gall most gall-inducing insects are limited to a single host species and in most cases also limited to a specific tissue type (e.g. leaf buds, catkins, stems, roots, etc) (Shorthouse & Rohfritsch, 1992). An intriguing question in the study of species interactions is how an organism can repurpose existing genes/pathways to manipulate the growth and structure of another organism from a different kingdom of life to create a unique and complex structure. Even though this question dates back to scholarly writings in ancient Greece and China (Redfern, 2011), we still know very little about how these manipulative interactions evolved, the genes or gene products involved, or whether the regulatory mechanism(s) behind gall induction and development is consistent across independent insect lineages that induce galls and their host plants.

Gall induction has evolved independently in six insect orders and has been described for >13,000 insect species (Espírito-Santo & Fernandes, 2007). These include gallers that cause substantial reductions in yield on the agriculture crops grape (*Vitis*), wheat (*Triticum*), blueberry (*Vaccinium*) and rice (*Oryza*) and are considered significant crop pests (Granett, Walker, Kocsis, & Omer, 2001; Smiley, Gourlie, Whittaker, Easley, & Kidwell, 2004; Way, Grigarick, Litsinger, Palis, & Pingali, 1991). Cynipid wasps (Hymenoptera: Cynipidae) comprise one of the most charismatic and phenotypically variable groups of gallers. Cynipid species are diverse and distributed worldwide, with over 800 species in North America alone (Espírito-Santo & Fernandes, 2007). Most gall inducing cynipid species are cyclically parthenogenetic and their sexual and asexual generations utilize different host tissues and induce unique gall morphologies (Redfern, 2011). This study focuses on *Dryocosmus quercuspalustris* , the succulent oak gall wasp, which can successfully gall several species of red oak (section Lobatae).

The sexual generation of *D. quercuspalustris* mainly galls leaf tissue and, more rarely, can successfully gall catkins and leaf buds (Redfern, 2011). The galls form a unique spherical structure not normally found on the host plant, which houses the developing cynipid larvae (Fig. 1a). Gall initiation occurs during the spring leaf flush, where the wasp inserts (i.e., oviposits) a single egg, as well as a maternal secretion from the venom gland, into meristematic tissue of the host plant. The gall develops into a hollow spherical outer capsule and an inner capsule suspended by connective tissues (Fig. 1c). The inner gall tissue contains a thick layer of nutritive cells derived from parenchyma (meristematic ground tissue that generally makes up the soft parts of plants) that feeds the developing larva (Csóka, Stone, & Melika, 2005) (Fig. 1d). The nutritive galls cells typically consist of enriched cytoplasm, fragmented vacuoles, and abundant cell organelles with elevated levels of carbohydrates, lipids, soluble sugars and proteins (Raman, 2011). Once the larva stops feeding, the connective tissue supporting the inner capsule disappears, leaving the inner capsule to freely move within the outer capsule, leading to a second common name, the roly-poly gall (Fig. 1e&f). This feature may function in anti-parasitoid defense. While previous histological studies have shown that there are distinct tissue types in galls (Raman, 2011), previous transcriptomic studies of galled tissue have combined all tissues into a single sample, potentially masking important tissue-specific gene expression patterns. The extreme separation of the inner and outer tissue types in *D. quercuspalustris* galls gives us the opportunity to confidently separate the distinct tissue types and identify tissue-specific genes and pathways.

To determine how *D. quercuspalustris* changes the gene expression profile of its host and identify tissue-specific genes and pathways involved in this interaction, we performed a *de novo*transcriptomic analysis of the wasp and the succulent oak gall on Northern Red Oak (*Quercus rubra*) during the active growth stage of gall formation. Gene expression profiles were constructed from outer capsule gall tissue, inner capsule gall tissue with the wasp larvae removed, and connected ungalled leaf tissue, to examine the plant's perspective of gall development and growth. From these transcriptomes we are able to address the following questions: 1) How closely do galled tissues resemble the plant tissue from which they arose (i.e. is this gall a unique parasite-specific organ or a modified plant organ)? 2) How distinct are different tissues within the gall and how do their gene expression profiles differ? and 3) Based on previous work from other galling systems, how conserved are differentially expressed genes and pathways in different galling guilds? By applying the powerful tool of transcriptomic sequencing, combined with a comparative analysis across taxa, we are now able to start to uncover the molecular ecology of this complex interspecies relationship and address the

evolution of gall induction and development.

Materials and Methods

Collection and RNA extraction. Samples for this study were collected from the University of Rochester campus, Rochester NY in June 2014 (replicate 1) and 2015 (replicate 2 and 3) from different individual trees. Galls caused by *D. quercuspalustris* on *Q. rubra* were collected during the growth phase of gall development. The growth phase was determined by the presence of live tissue connecting the inner and outer capsules, a thick layer of nutritive tissue within the inner capsule, and an actively feeding wasp larva. Leaves containing galls were removed from the tree and taken to the lab to be dissected. Using sterile razor blades and microforceps, three tissue types were collected from the a single gall per replicate: 1) a ˜10mm band of leaf tissue that was attached to the gall, 2) outer cortex tissue, including the tissue that connects to the inner cortex, and 3) inner cortex with the wasp larva removed (Fig. 2a). Samples were stored in 1.5ul microcentrifuge tubes in RNAlater (Qiagen) for plant samples and stored at -80°C. For all three replicates, the three tissue types were paired samples all belonging or attached to the same individual gall.

Total RNA extractions for the plant samples were performed with the Spectrum Plant Total RNA Kit (Sigma). The quantification and quality of the samples were checked using Agilent 2100 Bioanalyzer. TruSeq mRNA (Illumina) library construction and 100bp paired-end sequencing on Illumina HiSeq 2500 platform were performed by University of Rochester Genomics Research Center (URGRC). cDNA for each species was indexed with a unique adapter. Each library was normalized by equimolar multiplexing before sequencing at ˜1 library/$10^{\text{th}}$of a lane.

De novo **transcriptome assembly and filtering.**Pre-processing of raw reads included seqClean adaptor, uniVec database filtering, and poly-A tail trimming, and quality trimming using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) (Pearson, Wood, Zhang, & Miller, 1997) and quality was assesed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc). These are available at (Martinson, Werren, & Egan, 2018). *De novo*transcriptomes for oak was assembled in Trinity v.r2013-02-25 (Grabherr et al., 2011). The *Q. rubra de novo* transcriptome was assembled from combining all the reads from the leaf, inner capsule, and outer capsule samples from the first replicate.

Assemblies were first filtered by Open Reading Frame (ORF) prediction. Two separate programs were used: Transdecoder (Haas et al., 2013) with a word size minimum of 60bp and ORFPredictor (Min, Butler, Storms, & Tsang, 2005) with default settings. Replicates of the same ORF found in both programs (99% nucleotide sequence similarity) were removed using USEARCH v.7.0 (Edgar, 2010). Processed reads were mapped to ORF-filtered contigs using Burrows-Wheeler Aligner (BWA v.0.7.8) allowing for two mismatches per raw read (-n 2) (Li & Durbin, 2009). Fragments Per Kilobase Per Million (FPKM) values were calculated in Cufflinks v.2.2.0 (Trapnell et al., 2010). As a second filter, any contig with the three replicates averaging <10 FPKM across all tissues were not included in further analyses.

Statistical Analysis . DESeq v1.3.17 was used to generate normalized read counts for each sample and differential expression calls for each tissue type (Love, Huber, & Anders, 2014). In all comparisons, three biological replicates of each tissue were used to calculate significant differential expression. To be considered significantly differentially expressed a contig needed an adjusted p-value < 0.01 with DEseq2 (Love et al., 2014). For functional annotation, all transcripts were searched against the non-redundant protein database (nr) using BLASTx with a cutoff e-value $< 1 \times 10^{-5}$. Gene ontology (GO) terms were then assigned by BLAST2GO v2.5.0 (Conesa et al., 2005). Significantly overrepresented gene ontology (GO) categories were determined using BiNGO in Cytoscape with an adjusted p-value < 0.01 (Maere, Heymans, & Kuiper, 2005).

Multidimensional scaling analysis. To compare the expression profiles between tissue types, galled tissues were compared to previously published *Glycine max* profiles from young leaves, flowers, roots, nodules, pod shells (1cm and 14d), and seeds (10d, 21d, 28d, and 42d)(Severin et al., 2010). The soybean was chosen, because at the time of the analysis it was the closest related plant species with tissue specific transcriptome data. One-to-one orthologs were determined between the soybean and oak gene sets by reciprocal best BLASTn with a cutoff e-value $< 1 \times 10^{-5}$. The count data from 11,186 genes were used to

conduct a multidimensional scaling analysis in EdgeR (Robinson, McCarthy, & Smyth, 2010). Heatmaps were generated using Heatmapper with average linkage clustering and the Pearson distant measurement method (Babicki et al., 2016).

Results and Discussion

Here we present the first study that looks at gene expression profiles of different tissue types within a gall, which uncovers important tissue specific gene expression patterns that would have been masked by pooling. We determined the expression profiles for normal leaf tissue, outer gall tissue, and inner gall tissue (with the wasp larvae removed) from the succulent oak gall of *D. quercuspalustris* on *Q. rubra* , to determine the extent to which the wasp manipulates gene expression profiles and to identify the genes and pathways involved in the formation of the gall.

Succulent oak gall assembly and expression profiles

The *de novo* assembly of the *Q. rubra* transcriptome resulted in 83,781 contigs, which was reduced to 34,687 contigs after filtering for the presence of open reading frames (ORFs) and minimum expression cutoffs. Approximately 60% of the filtered transcriptome was assigned an annotation from the NCBI nr database using BLASTx with a e-value $<10^{-5}$ cutoff. All three tissue types had similar number of total genes expressed (with average expression >1 fragments per kilobase of transcript per million (FPKM) across replicates) ranging from 29,750 in the inner gall tissue to 33,392 in the leaf. The inner and outer galled tissue also had similar numbers of genes specific to one tissue type (average <1 FPKM in both other tissues) with 289 genes specifically expressed in the inner gall and 282 specific genes in the outer gall; the leaf tissue had significantly higher specific expression with 658 genes only expressed in normal leaf tissue.

Differences among the three tissue types were clear, as the expression profiles of the three independently sampled replicates of each tissue type formed three distinct clusters (Fig. 2b). As replicates were collected across multiple years from different host trees, this demonstrates that gene expression changes induced by the cynipid wasps are very consistent. Comparing the expression profiles of the three oak tissues to previously published soybean tissues (Severin et al., 2010), we surprisingly find that the gene expression profiles of a soybean flower and root are more closely related than the inner gall tissue to the leaf tissue from which it was derived. In fact, the inner gall gene expression profile did not closely align with any plant tissue in our analysis, in contrast to the oak and soybean leaf expression profiles being quite similar to each other. The expression profile of the outer gall tissue is distinct from the inner gall, indicating that it is a unique tissue type and is more similar to the leaf than the inner gall tissue.

This analysis shows that cynipid wasps are creating an entirely new and complex organ from its oak host tissue that contains multiple distinct tissue types. The comparison of the expression profiles of the three tissues indicates that the inner gall tissue is best described as a unique parasite specific tissue, whereas the outer gall tissue is more reflective of a modified plant leaf. The differences between tissue types are also supported by the number of significantly differentially expressed genes between each tissue (Fig. 2c). Compared to the inner gall tissue, approximately 20% (6783 contigs) of the outer gall tissue and 28% (9670 contigs) of the leaf tissue contigs are significantly differentially expressed. Whereas the leaf and outer gall differ in only ˜5% (1996 contigs) of the transcriptome (Fig. 2d).

This possible hybrid mix of parasite-specific and plant-modified tissues is likely to be true of other galling insects as well. Previous studies have noted that the inner tissues of insect galls are far more histologically similar to each other than their outer surfaces (Bronner, 1992; Nyman & Julkunen-Tiitto, 2000). It could be proposed that since the outer tissue is further away from the source of the galling stimulus (i.e. the chewing larva), the outer tissue may be more bound to the gene expression of the host plant and specific plant tissue from which the gall originates, however more studies are needed to test this hypothesis.

Comparisons to soybean tissues also allows us to contrast the expression profiles of insect-induced galls to bacteria-induced galls. Root nodules in soybeans are relatively simple growths caused by symbiotic nitrogen-fixing bacteria (rhizobia) and initiated by a small set of*nod* bacterial genes (Kosslak, Bookland,

Barkei, Paaren, & Appelbaum, 1987). The gene expression profile of a root nodule is very close to the expression of a soybean root, suggesting that a rhizobium nodule more resembles a modified-root than a bacteria-specific organ. The difference between the inner gall and oak leaf far exceeds the difference between the soybean root and root nodule, which more closely resembles the distance between the outer gall and leaf. (Severin et al., 2010). The complexity of the wasp gall over a rhizobium nodule could suggest that the mechanism behind the wasp gall is also more complex, involving multifaceted signaling from the ovipositing female and feeding larvae, and involving many wasp genes.

Differentially expressed in both galled tissues

Genes that are significantly differentially regulated in both inner and outer gall tissue compared to leaf tissue may represent genes necessary for gall growth and development. Only 319 genes have significantly higher expression in both inner and outer tissue compared to leaf tissue. The main groups of genes can be broken down into metabolism and defense. Metabolism genes with the greatest increase in expression were involved in sugar metabolism and transport (22 total), amino acid biosynthesis (16 genes), and ethylene biosynthesis (7 genes). Increases in sugar metabolism and transport indicate that galled tissue has shifted from an autotrophic to a heterotrophic nutrition source and has become a nutrition sink on the host plant. Increases in ethylene production have been shown previously in wasp, mite, aphid, and sawfly galls, with the hypothesis that it defending the gall against herbivores and pathogens (Hearn et al., 2019; Samsone, Andersone, & Ievinsh, 2012), however polyphenol oxidase and peroxidase activity were shown to decrease in galled tissue (Gailite, Andersone, & Ievinsh, 2005). Additionally, in this study 1-aminocyclopropane-1-carboxylate oxidase (ACO), which catalyzes the synthesis of ethylene, shows 151-fold increase in expression in the inner gall tissue and a 31-fold increase in the outer gall tissue, which doesn't support a defensive role as it would directly impact the feeding wasp larvae. Ethylene is also an endogenous plant grow regulator and is important in fruit ripening. Previous studies have shown that galling insects activate genes involved in flower and fruit development (Schultz, Edger, Body, & Appel, 2019). The patterns of ACO expression in the inner and outer tissue therefore may support the hypothesis that ethylene biosynthesis plays a larger role in gall growth rather than defense.

At least 21 genes that do correspond to defense responses were up regulated in both inner and outer gall tissue. The largest responders are pgip with a 65-fold and 125-fold increase in inner and outer, respectively compared to leaf tissue and defensin (40-fold and 8-fold increase), both of which are antifungal peptides (Ai-Guo et al., 2000; Powell et al., 2000). Several studies have shown that insect galls can affect the composition of fungal endophyte species in plant tissues and galling insects can introduce fungi to their host plants (Batra & Lichtwardt, 1963; Lawson, Christian, & Abbot, 2014; Martinson, Herre, Machado, & Arnold, 2012; Wilson, 1995). A previous study of two cynipid and one aphid galls showed that 12.5% of the larvae surveyed die as a result of an invasion by a fungal endophyte (Wilson, 1995). Therefore, these proteins could be playing the important role of inhibiting the growth of fungi naturally occurring in the host plant or introduced by the wasp that could invade the nutritive-rich galled tissue. It would be detrimental, however, to have the plant's anti-herbivore response be triggered by the feeding wasp. The gene that encodes the DNA-binding protein ESCAROLA, which can negatively regulate plant innate immunity (Lu, Zou, & Feng, 2010), was also highly up-regulated and may be a possible mechanism the wasps employ to evade plant defenses.

Another significantly up-regulated gene in both inner and outer gall tissue was *early nodulin-93* (*ENOD93*), which is also involved in the induction of root nodules by rhizobia bacteria (Kosslak et al., 1987; You et al., 2003). ENOD genes were also found to be up-regulated in another cynipid/oak galling system (*Biorhiza pallida* /*Quercus robur*), where it has been proposed that somatic embryogenesis is induced by wasp GH18 chitinases cleaving the oak early nodulin genes (Hearn et al., 2019). The expression of ENOD genes in the legume-rhizobia system occurs after the exchange of biochemical signals between the plant and bacteria. The first signal, called the inducer, is typically a flavonoid released by the plant. This stimulates the bacteria to release *nod* genes, which induces the expression of ENOD genes by the plant to initiate nodule growth. Flavonoids are produced through the phenylopropoanoid-acetate pathway, and genes involved in this pathway have been found to be upregulated in several galling systems. In the fig-fig wasp mutualism,

all the genes necessary to produce flavonoids were significantly upregulated in the gall flowers (receptive to gall formation) compared to seed flowers (unreceptive to gall formation) prior to the fig wasp entering the fig (Martinson, Hackett, Machado, & Arnold, 2015). The difference in flavonoid profiles could be a possible signal to the fig wasp to oviposit into gall-receptive tissues. Abrahamson et al. (2003) demonstrated that six species of sympatric *Quercus* species have unique profiles of leaf phenolics and the differences were strongly correlated with the host specificity of galling cynipids, indicating that the oak's chemistry profile influences the cynipid host range (Abrahamson, Hunter, Melika, & Price, 2003). The phenylopropoanoid-acetate pathway is also upregulated in the *D. quercuspalustris* /oak system, however only in the outer gall tissue (see below). The overlap of these genes raise the exciting possibility that some genes mediating tissue growth between plant-biotic interactions may be conserved between bacteria and cynipid wasps.

There are 593 genes that are significantly down-regulated in both the inner and outer gall tissue compared to leaf tissue. The largest group (58 genes) is involved in photosynthesis (Fig. 3a). Most studies investigating the photosynthetic capacities of leaf galls have found significantly lower photosynthetic rates in galls than in the surrounding leave tissue and are mostly regarded as strong carbon sinks (Andersen & Mizell III, 1987; Huang et al., 2014; Jiang, Veromann-Jurgenson, Ye, & Niinemets, 2018; Larson & Whitham, 1991). However, some galls substantially contribute to photosynthesis, hereby reducing their impact on the host (Dorchin, Cramer, & Hoffmann, 2006). The inner gall tissue of *D. quercuspalustris* , shows little evidence of photosynthetic activity, with photosynthesis genes expressed at only 5% of the levels found in leaf tissue. This is unsurprising since the inner gall tissue is not exposed to light and is white in color. The outer gall tissue, which is green in color, expresses photosynthesis genes at a 26% level, which might suggest that the outer gall tissue is photosynthetically active and contributes some to the growth and maintenance of the gall, but probably is still an overall sink to the host plant.

Inner gall tissue gene expression

Genes differentially expressed in the inner capsule of the gall (compared to both outer and leaf tissue) are important for elucidating the galling mechanism because this structure is the interface of the plant and the feeding larva (Csoka et al., 2005). Genes up-regulated in the inner gall capsule suggest that these cells are transcriptionally and translationally active, including many subunits of DNA-directed RNA polymerase, spliceosomal genes, and eukaryotic translation initiation factors, which may indicate rapid cell division or growth. Additionally, there are many genes expressed for the folding and transporting of proteins, including all the genes necessary to form nuclear pore complexes to transfer proteins across the nuclear envelope, which indicates a dynamic cytoplasm-nucleus interaction is needed for this interaction.

Histological studies in cynipid galls have shown a starch gradient in the nutritive tissues, in which the concentrations of starch are highest near the edge of the inner gall tissue and decreases as it approaches the feeding larva (Bronner, 1992). If the larva is killed, starch begins to appear equally throughout the inner gall tissue, which indicated the active degradation of starch (Bronner, 1992). While previous studies have noted high levels of the starch degrading enzyme amylase in an opposite gradient to starch (Bronner, 1992), this transcriptome found no high expressing amylases or other starch degrading enzymes in the inner gall tissue. This would suggest that the feeding larvae is solely providing the enzymes for starch degradation.

The oak is contributing to the starch gradient in that the highest-expressing, upregulated gene in the inner tissue is sucrose synthase 2 (11709 FPKM), which cleaves sucrose to provide UDP-glucose and fructose to several metabolic pathways, specifically starch synthesis. Gall tissues have previous been shown to have higher levels of sugar than normal plant tissue, it is thought that these sugars are transported to the gall tissue in the form of sucrose (Bronner, 1992).The other main enzyme for sucrose degradation are invertases, which previous studies have shown in high abundances in galls (Bronner, 1992; de Oliveira & dos Santos Isaias, 2010; Ruan, Chen, Yang, & Wang, 2017). However, no invertases shows an upregulation in the inner gall tissue in the current study, which suggests that sucrose synthase 2 is the primary plant-derived enzyme. This may be unique to *Q. robur*or invertases may again have been insect-derived enzymes. The high levels of sugars are also supported by the up-regulation of the genes involved in glycolysis and the TCA cycle. However, the gluconeogenesis-specific gene phosphoenolpyruvate carboxykinase is also upregulated, which

suggests that glycolysis genes are being used to covert pyruvate, lactate, or glycerol into glucose to feed the wasp larva as well (Fig. 3b).

Signaling from the actively chewing larvae develop and maintain plant galls (Stone, Schonrogge, Atkinson, Bellido, & Pujade-Villar, 2002), though the nature of the signals are still unknown. One hypothesis is that wasps use the plant's RNA interference mechanisms (RNAi) to modify plant gene expression (Oates, Denby, Myburg, Slippers, & Naidoo, 2016). Our transcriptome shows some evidence for this with overrepresented gene ontology (GO) terms in the 1656 significantly up-regulated inner capsule genes for gene silencing, RNA methylation, RNA modification, and mRNA transport (Fig. 4a). Genes in the RNA$i$pathway that are upregulated in the inner gall capsule include argonaute 16 and endoribonuclease Dicer. Nine F-box proteins are also significantly upregulated and are represented in gene networks broadly regulated by microRNA-mediated gene silencing via RNAi(Jones-Rhoades, Bartel, & Bartel, 2006). Other mechanisms that repress transcription that are up-regulated include histone deacetylases, SIN3 genes, and methyltransferases.

An obvious next question for the RNAi hypothesis is "Which genes are being repressed/silenced?" A total of 3566 genes are significantly down-regulated in the inner capsule compared to leaf tissue with the largest category of down-regulated genes, outside of photosynthesis, being genes related to defense and stress response (Fig. 4b). Genes involved in jasmonic, salicylic, and abscisic acid pathways are significantly down-regulated in the inner gall tissue. Chewing insects, such as cynipid larvae, usually trigger the jasmonate pathway to induced defense responses (Walling, 2000), which suggests that some mechanism is suppressing the oak's defense response. Additionally, at least 44 leucine-rich repeat family proteins, 15 disease resistance protein, 9 TMV resistance protein, which detect pathogens and triggers the defense response, show reduced expression (Martin, Bogdanove, & Sessa, 2003; Padmanabhan, Cournoyer, & Dinesh-Kumar, 2009). Overall large parts of the plant innate immune system are suppressed in the inner capsule of the gall, with the exception of antimicrobial defensive genes mentioned above. Another large category of down-regulated genes is related to aromatic compound metabolism, specifically flavonoid metabolic processes, which shows significant up-regulation in the outer gall tissue.

Outer gall tissue gene expression

Whereas there was no pattern in the 25 significantly down-regulated outer gall genes when compared to both inner and leaf tissue, many of the 400 significantly up-regulated genes are involved in various plant defense systems. This has previously been seen in other galling species. Nyman and Julkunen-Titto (2000) showed that sawflies induced elevated levels of tannins, flavonols, other flavonoids, and salicylates in the outer tissue of willow galls compared to the gall interior or ungalled leaves (Nyman & Julkunen-Tiitto, 2000) and Ikai and Hijii (2007) showed the same pattern for just tannins in cynipid/*Quercus* galls (Ikai & Hijii, 2007). *Dryocosmus quercuspalustris* induces all the genes required for flavonoid biosynthesis, thaumatins, chitinases, and many genes involved in the salicylic acid pathway (e.g. salicylic acid-binding protein 2) to be expressed specifically in the outer gall tissue away from the feeding wasp larva. These defense responses are consistent with the predicted functional role of the outer gall tissue, which is to provide protection to the nutritive-rich, less defended inner tissue and the feeding larva from bacteria, fungi, and parasitoid wasps. Genes involved in the protection against oxidative stress and the shikimate and phenylpropanoid biosynthesis pathways were also highly up-regulated. Some of the highest expressing genes are the five main genes at the start of the phenylopropoanoid acetate pathway (phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, coumarate CoA ligase, chalcone synthase, and chalcone isomerase) with >1000 FPKM in the outer gall tissue, significantly higher than in both the inner gall and leaf. This phenolics may be playing a defensive role, as they have inhibitory activity against microbes (Galeotti, Barile, Curir, Dolci, & Lanzotti, 2008) and in other plant-insect interactions, phenolics can be feeding deterrents by decreasing the plants digestibility or acting as toxins (Mierziak, Kostyn, & Kulma, 2014; Simmonds, 2001). These chemicals may also be playing an important signaling role in the interactions between the wasp and oak, similar to the flavonoids in the rhizobium/legume system. Identifying the exact phenolic profile in the oak leaves and gall tissue and tracking how it changes over time are important next steps in determine the role they play in gall induction and ecology.

Many studies have proposed that the phytohormone auxin, specifically indole acetic acid (IAA), may be involved in gall induction. Increased levels of IAA have been found in galled tissue and in the galling insects themselves in several systems (reviewed in (Tooker & Helms, 2014)). However, previous studies looking at cynipid gallers on Chestnut (Wood & Payne, 1988) and oak (Hearn et al., 2019) hosts found little evidence for the involvement of auxin and IAA in cynipid galls. The current study shows the up-regulation of indole-3-acetic acid-amido synthetase and six auxin response factors. However, transcription levels of these genes are relatively low and there are significantly more down-regulated auxin genes with at least 51 genes related to the synthesis, transport, and response to auxin. This provides further evidence that auxin and IAA probably is not a primary mechanism in the cynipid wasp system, however it could be that the role of auxin is not reflected in gene expression changes and further analyses of hormone levels in cynipid galls will be needed to fully assess its role.

Comparison to agricultural galling pests

How galls are induced is known in a handful of organisms (e.g.*nod* genes in Rhizobia or the insertion of T-DNA in*Agrobacterium tumefaciens*), however the mechanism behind gall induction is still unknown in any of the more complex galls created by insects. Gall induction has evolved independently in many insect groups, but it is not known if insect gallers use the same or similar mechanisms. While many aspects of gall metabolism seem to be shared across some galling guilds (examples above), the transcriptomes of galled plant tissue have not yet been directly compared. Previously published transcriptomes on phylloxera gallers on grapes (Nabity, Haus, Berenbaum, & DeLucia, 2013), gall midge on rice (Rawat, Neeraja, Nair, & Bentur, 2012), and the Hessian fly on wheat (Liu et al., 2007) allow us to compare and contrast to the differentially expressed genes in cynipid wasp galls. In all four galling systems, genes involved in photosynthesis are down-regulated in galled tissue and genes involved in glycolysis, carbohydrate metabolism, sugar transport, and amino acid metabolism are up-regulated. Genes involved in antimicrobial defensive are found to be up-regulated in all systems; specifically, the antifungal gene defensin is up-regulated in both oak and wheat galls. Interestingly, the above systems all show differential expression in phenylpropanoid metabolism. Many phenylpropanoids show antimicrobial activity, so it may be only protecting the gall from pathogens, however phenylpropanoids can also have regulatory roles such as modulators of cell division and auxin transport, which may suggest a more fundamental role in gall growth (Dixon et al., 2002). Ethylene signaling is also up-regulated in wheat, grape, and oak galls. This overlap supports the hypothesis that there are underlying mechanisms of gall development even in disparate plants and galling guilds.

In conclusion, *D. quercuspalustris* induces large gene expression changes in oaks. Insect galls contain distinct tissue types that have extremely different expression profiles, with the inner gall classified as unique parasite-specific tissue and the outer gall tissue being more reflective of a modified plant leaf. It will be important for future gall research to evaluate these tissues separately to avoid cancelling out or overwhelming important signals by pooling disparate tissues together. From separating these tissues, we have found up-regulation in genes involved in gene silencing and modification and changes in plant metabolism that may give insights into how wasps manipulate plants. By comparing our results to previous gall studies, we found that many affected genes and pathways are shared with other distant galling guilds, possibility as distant as galling bacteria. These shared pathways will be an important focus in future research to determine if galling insects share common mechanisms to manipulate plants.

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References

Abrahamson, W. G., Hunter, M. D., Melika, G., & Price, P. W. (2003). Cynipid gall-wasp communities correlate with oak chemistry.*Journal of Chemical Ecology, 29* (1), 209-223.

Agrawal, A. A., Ackerly, D. D., Adler, F., Arnold, A. E., Caceres, C., Doak, D. F., . . . Werner, E. (2007). Filling key gaps in population and community ecology. *Frontiers in Ecology and the Environment, 5* (3),

145-152. doi:10.1890/1540-9295(2007)5[145:Fkgipa]2.0.Co;2

Ai-Guo, G., Hakimi, S. M., Mittanck, C. A., Wu, Y., Woerner, B. M., Stark, D. M., . . . Rommens, C. M. (2000). Fungal pathogen protection in potato by expression of a plant defensin peptide. *Nature biotechnology, 18* (12), 1307.

Andersen, P., & Mizell III, R. (1987). Physiological effects of galls induced by *Phylloxera notabilis* (Homoptera: Phylloxeridae) on pecan foliage. *Environmental Entomology, 16* (1), 264-268.

Babicki, S., Arndt, D., Marcu, A., Liang, Y., Grant, J. R., Maciejewski, A., & Wishart, D. S. (2016). Heatmapper: web-enabled heat mapping for all. *Nucleic Acids Research, 44* (W1), W147-W153.

Batra, L. R., & Lichtwardt, R. W. (1963). Association of fungi with some insect galls. *Journal of the Kansas Entomological Society, 36* (4), 262-278.

Bronner, N. (1992). The role of nutritive cells in the nutrition of cynipids and cecidomyiids. In *Biology of Insect-Induced Galls*(pp. 118-140).

Bronstein, J. L., Alarcon, R., & Geber, M. (2006). The evolution of plant–insect mutualisms. *New Phytologist, 172* (3), 412-428. doi:10.1111/j.1469-8137.2006.01864.x

Conesa, A., Gotz, S., Garcia-Gomez, J. M., Terol, J., Talon, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research.*Bioinformatics, 21* (18), 3674-3676. doi:10.1093/bioinformatics/bti610

Csoka, G., Stone, G., & Melika, G. (2005). Biology, ecology, and evolution of gall-inducing Cynipidae. In *Biology, ecology and evolution of gall-inducing arthropods* (Vol. 2, pp. 573-642).

Dawkins, R. (1999). *The Extended Phenotype : the Long Reach of the Gene* . New York: Oxford University Press

de Oliveira, D. C., & dos Santos Isaias, R. M. (2010). Cytological and histochemical gradients induced by a sucking insect in galls of*Aspidosperma australe* Arg. Muell (Apocynaceae). *Plant Science, 178* (4), 350-358.

Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S., & Wang, L. (2002). The phenylpropanoid pathway and plant defence—a genomics perspective. *Molecular plant pathology, 3* (5), 371-390.

Dorchin, N., Cramer, M. D., & Hoffmann, J. H. (2006). Photosyntheis and sink activity of wasp-induced galls in *Acacia pycnantha* .*Ecology, 87* (7), 1781-1791. doi:10.1890/0012-9658(2006)87[1781:PASAOW]2.0.CO;2

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26* (19), 2460-2461.

Espirito-Santo, M., & Fernandes, G. W. (2007). How many species of gall-inducing insects are there on earth, and where are they?*Annals of the Entomological Society of America, 100* (2), 95-99.

Gailite, A., Andersone, U., & Ievinsh, G. (2005). Arthropod-induced neoplastic formations on trees change photosynthetic pigment levels and oxidative enzyme activities. *Journal of Plant Interactions, 1* (1), 61-67.

Galeotti, F., Barile, E., Curir, P., Dolci, M., & Lanzotti, V. (2008). Flavonoids from carnation (Dianthus caryophyllus) and their antifungal activity. *Phytochemistry Letters, 1* (1), 44-48.

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., . . . Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature biotechnology, 29* (7), 644–652.

Granett, J., Walker, M. A., Kocsis, L., & Omer, A. D. (2001). Biology and management of grape phylloxera. *Annual review of entomology, 46* (1), 387-412.

Haas, B. J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P. D., Bowden, J., . . . Regev, A. (2013). *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols, 8* (8), 1494-1512.

Hearn, J., Blaxter, M., Schonrogge, K., Nieves-Aldrey, J.-L., Pujade-Villar, J., Huguet, E., . . . Stone, G. N. (2019). Genomic dissection of an extended phenotype: Oak galling by a cynipid gall wasp.*PLoS genetics, 15* (11), e1008398.

Herre, E. A., Knowlton, N., Mueller, U. G., & Rehner, S. A. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology & Evolution, 14* (2), 49-53. doi:https://doi.org/10.1016/S0169-5347(98)01529-8

Huang, M. Y., Huang, W. D., Chou, H. M., Lin, K. H., Chen, C. C., Chen, P. J., . . . Yang, C. M. (2014). Leaf-derived cecidomyiid galls are sinks in Machilus thunbergii (Lauraceae) leaves. *Physiologia plantarum, 152* (3), 475-485.

Ikai, N., & Hijii, N. (2007). Manipulation of tannins in oaks by galling cynipids. *Journal of forest research, 12* (4), 316-319.

Jiang, Y., Veromann-Jurgenson, L. L., Ye, J., & Niinemets, U. (2018). Oak gall wasp infections of Quercus robur leaves lead to profound modifications in foliage photosynthetic and volatile emission characteristics. *Plant, cell & environment, 41* (1), 160-175.

Johnson, N., Graham, J., & Smith, F. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist, 135* (4), 575-585. doi:doi:10.1046/j.1469-8137.1997.00729.x

Jones-Rhoades, M. W., Bartel, D. P., & Bartel, B. (2006). MicroRNAs and their regulatory roles in plants. *Annu. Rev. Plant Biol., 57* , 19-53.

Kosslak, R. M., Bookland, R., Barkei, J., Paaren, H. E., & Appelbaum, E. R. (1987). Induction of *Bradyrhizobium japonicum* common*nod* genes by isoflavones isolated from *Glycine max* .*Proceedings of the National Academy of Sciences, 84* (21), 7428-7432.

Larson, K. C., & Whitham, T. G. (1991). Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions.*Oecologia, 88* (1), 15-21.

Lawson, S. P., Christian, N., & Abbot, P. (2014). Comparative analysis of the biodiversity of fungal endophytes in insect-induced galls and surrounding foliar tissue. *Fungal Diversity, 66* (1), 89-97.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics, 25* (14), 1754-1760.

Liu, X., Bai, J., Huang, L., Zhu, L., Liu, X., Weng, N., . . . Chen, M.-S. (2007). Gene expression of different wheat genotypes during attack by virulent and avirulent Hessian fly (*Mayetiola destructor*) larvae. *Journal of chemical ecology, 33* (12), 2171-2194.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology, 15* (12), 550.

Lu, H., Zou, Y., & Feng, N. (2010). Overexpression of AHL20 negatively regulates defenses in *Arabidopsis* . *Journal of integrative plant biology, 52* (9), 801-808.

Maere, S., Heymans, K., & Kuiper, M. (2005). BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics, 21* (16), 3448-3449.

Martin, G. B., Bogdanove, A. J., & Sessa, G. (2003). Understanding the functions of plant disease resistance proteins. *Annual review of plant biology, 54* (1), 23-61.

Martinson, E. O., Hackett, J. D., Machado, C. A., & Arnold, A. E. (2015). Metatranscriptome analysis of fig flowers provides insights into potential mechanisms for mutualism stability and gall induction. *PloS One, 10* (6), e0130745.

Martinson, E. O., Herre, E. A., Machado, C. A., & Arnold, A. E. (2012). Culture-free survey reveals diverse and distinctive fungal communities associated with developing figs (*Ficus spp.*) in Panama. *Microbial Ecology, 64* (4), 1073-1084.

Martinson, E. O., Kelkar, Y. D., Chang, C.-H., & Werren, J. H. (2017). The evolution of venom by co-option of single-copy genes. *Current Biology, 27* (13), 2007-2013. e2008.

Martinson, E. O., Werren, J.H., Egan, S.P. (2018) Cynipid wasp gall on *Quercus rubra* Raw sequence reads; NCBI, PRJNA490752.

Martinson, E. O., Wheeler, D., Wright, J., Siebert, A. L., & Werren, J. H. (2014). *Nasonia vitripennis* venom causes targeted gene expression changes in its fly host. *Molecular Ecology, 23* (23), 5918-5930.

Mierziak, J., Kostyn, K., & Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. *Molecules, 19* (10), 16240-16265.

Min, X. J., Butler, G., Storms, R., & Tsang, A. (2005). OrfPredictor: predicting protein-coding regions in EST-derived sequences. *Nucleic Acids Research, 33* , W677-W680.

Nabity, P. D., Haus, M. J., Berenbaum, M. R., & DeLucia, E. H. (2013). Leaf-galling phylloxera on grapes reprograms host metabolism and morphology. *Proceedings of the National Academy of Sciences, 110* (41), 16663-16668.

Nyman, T., & Julkunen-Tiitto, R. (2000). Manipulation of the phenolic chemistry of willows by gall-inducing sawflies. *Proceedings of the National Academy of Sciences, 97* (24), 13184-13187.

Oates, C. N., Denby, K. J., Myburg, A. A., Slippers, B., & Naidoo, S. (2016). Insect gallers and their plant hosts: from omics data to systems biology. *International journal of molecular sciences, 17* (11), 1891.

Padmanabhan, M., Cournoyer, P., & Dinesh-Kumar, S. (2009). The leucine-rich repeat domain in plant innate immunity: a wealth of possibilities. *Cellular microbiology, 11* (2), 191-198.

Pashalidou, F. G., Lambert, H., Peybernes, T., Mescher, M. C., & De Moraes, C. M. (2020). Bumble bees damage plant leaves and accelerate flower production when pollen is scarce. *Science, 368* (6493), 881-884. doi:10.1126/science.aay0496

Pearson, W. R., Wood, T., Zhang, Z., & Miller, W. (1997). Comparison of DNA sequences with protein sequences. *Genomics, 46* (1), 24-36.

Powell, A. L., van Kan, J., ten Have, A., Visser, J., Greve, L. C., Bennett, A. B., & Labavitch, J. M. (2000). Transgenic expression of pear PGIP in tomato limits fungal colonization. *Molecular Plant-Microbe Interactions, 13* (9), 942-950.

Raman, A. (2011). Morphogenesis of insect-induced plant galls: facts and questions. *Flora-Morphology, Distribution, Functional Ecology of Plants, 206* (6), 517-533.

Rawat, N., Neeraja, C. N., Nair, S., & Bentur, J. S. (2012). Differential gene expression in gall midge susceptible rice genotypes revealed by suppressive subtraction hybridization (SSH) cDNA libraries and microarray analysis. *Rice, 5* (1), 8.

Redfern, M. (2011). *Plant Galls* . Glasgow: Collins New Naturalist Library.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics, 26* (1), 139-140.

Ronquist, F., & Liljeblad, J. (2001). Evolution of the gall wasp-host plant association. *Evolution, 55* (12), 2503-2522.

Ruan, Z.-Y., Chen, X.-M., Yang, P., & Wang, B.-Y. (2017). Roles played by invertase and gene expression in the development of the horn-shaped gall on leaves of *Rhus chinensis* . *Functional Plant Biology, 44* (12), 1160-1170.

Samsone, I., Andersone, U., & Ievinsh, G. (2012). Variable effect of arthropod-induced galls on photochemistry of photosynthesis, oxidative enzyme activity and ethylene production in tree leaf tissues.*Environmental and Experimental Biology, 10* , 15-26.

Schultz, J. C., Edger, P. P., Body, M. J., & Appel, H. M. (2019). A galling insect activates plant reproductive programs during gall development. *Scientific Reports, 9* (1), 1-17.

Severin, A. J., Woody, J. L., Bolon, Y.-T., Joseph, B., Diers, B. W., Farmer, A. D., . . . Specht, J. E. (2010). RNA-Seq Atlas of*Glycine max* : a guide to the soybean transcriptome. *BMC plant biology, 10* (1), 160.

Shorthouse, J. D., & Rohfritsch, O. (1992). *Biology of Insect-Induced Galls* . Oxford: Oxford University Press.

Simmonds, M. S. (2001). Importance of flavonoids in insect–plant interactions: feeding and oviposition. *Phytochemistry, 56* (3), 245-252.

Smiley, R. W., Gourlie, J. A., Whittaker, R. G., Easley, S. A., & Kidwell, K. K. (2004). Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with Fusarium crown rot and lesion nematode. *Journal of economic entomology, 97* (2), 397-408.

Stone, G. N., & Schonrogge, K. (2003). The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution, 18* (10), 512-522.

Stone, G. N., Schonrogge, K., Atkinson, R. J., Bellido, D., & Pujade-Villar, J. (2002). The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual review of entomology, 47* (1), 633-668.

Tooker, J. F., & Helms, A. M. (2014). Phytohormone dynamics associated with gall insects, and their potential role in the evolution of the gall-inducing habit. *Journal of Chemical Ecology, 40* (7), 742-753.

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., . . . Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature biotechnology, 28* (5), 511–515.

Walling, L. L. (2000). The myriad plant responses to herbivores.*Journal of Plant Growth Regulation, 19* (2), 195-216.

Way, M. O., Grigarick, A. A., Litsinger, J. A., Palis, F., & Pingali, P. (1991). Economic thresholds and injury levels for insect pests of rice. In *Rice Insects: Management Strategies* (pp. 67-105): Springer.

Wheat, C. W., Vogel, H., Wittstock, U., Braby, M. F., Underwood, D., & Mitchell-Olds, T. (2007). The genetic basis of a plant–insect coevolutionary key innovation. *Proceedings of the National Academy of Sciences, 104* (51), 20427-20431. doi:10.1073/pnas.0706229104

Wilson, D. (1995). Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungal inquilines? *Oecologia, 103* (2), 255-260.

Wood, B., & Payne, J. (1988). Growth regulators in chestnut shoot galls infected with oriental chestnut gall wasp, *Dryocosmus kuriphilus*(Hymenoptera: Cynipidae). *Environmental Entomology, 17* (6), 915-920.

You, M. K., Hur, C. G., Ahn, Y. S., Suh, M. C., Jeong, B. C., Shin, J. S., & Bae, J. M. (2003). Identification of genes possibly related to storage root induction in sweetpotato. *FEBS Letters, 536* (1-3), 101-105.

Data availability. All the RNA-Seq raw reads generated during this study have been deposited in the NCBI SRA database under the accession PRJNA490752. The filtered and unfiltered *de novo* oak transcriptome assembly, overrepresented GO terms, differentially expressed gene lists and raw FPKM values for the heatmaps can be found at figshare [Reviewer private link: https://figshare.com/s/8b17d6061a3589f7cb71].

Author Contributions. EOM designed research, performed research, analyzed data, and wrote the paper. SPE and JHW analyzed data, and wrote the paper.

Figures and Tables

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Fig. 1 Development of a Succulent Oak Gall. A) Intact gall on oak leaf. B) *Dryocosmus quercuspalustris* female. C) A cross section of an actively growing gall with the intact inner capsule suspended by connective tissue. D) A cross section of an actively growing inner capsule with the wasp larva feeding on the thick layer of nutritive tissue. E) A cross section of a mature gall with an intact inner capsule freely moving after the connective tissue has disappeared. F) A cross section of a mature gall inner capsule showing a prepupal larva and the absence of nutritive tissue.

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Fig. 2 Changes in gene expression profiles in inner gall, outer gall, and leaf tissue. A) Illustration of the sampled tissue types with the inner gall tissue in purple, the outer gall tissue in red and the leaf tissue within the dotted line in green B) A multidimensional scaling analysis (MDS) comparing the expression profiles between inner gall, outer gall, and leaf oak tissue (green) and previously published soybean (*Glycine max*) profiles from young leaves, flowers, roots, nodules, pod shells (1cm and 14d), and seeds (10d, 21d, 28d, and 42d) (Severin et al., 2010) (purple). C) Significantly differentially expressed genes between inner/leaf, inner/outer, and outer/leaf tissue. Down-regulated genes shown in shaded area. D) Overlap of significantly differentially expressed genes among inner/leaf, inner/outer, and outer/leaf tissue comparisons.

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Fig. 3 Gene expression changes for photosynthesis and gluconeogenesis. Heat maps of all A) 159 genes assigned to the photosynthesis GO term and B) 113 genes assigned to the gluconeogenesis GO term as well as their child terms in the *Q. rubra*transcriptome for all three replicates of inner gall tissue, outer gall tissue, and normal leaf tissue that had >10 FPKM in at least one sample. Expression is label from lowest (purple) to highest (teal) in each row.

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Fig. 4 Gene expression changes for methylation and defense. A) Heat maps of the general category of RNA modification which contains the GO terms and their child terms for 1) RNA silencing (n=27), 2)

mRNA transport (n=72), and 3) RNA methylation (n=146) B) A heat map of all 433 genes assigned to the defense GO term in the *Q. rubra*transcriptome for all three replicates of inner gall tissue, outer gall tissue, and normal leaf tissue that had >10 FPKM in at least one sample. The highly expressed genes in the inner tissue include many genes for antimicrobial peptides. Expression is label from lowest (grey) to highest (red) in each row.