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Authors: Lauer, N. T., and Rossi, A. M.

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## EFFECTS OF MANUAL DAMAGE ON TURKEY OAK (FAGALES: FAGACEAE) FOLIAR TANNIN CONCENTRATION AND SUBSEQUENT HERBIVOROUS INSECT ABUNDANCE

N. T. LAUER AND A. M. ROSSI

Department of Biology, University of North Florida, Jacksonville, FL 32224

### ABSTRACT

Foliar tissue of turkey oak trees, *Quercus laevis* Walter (Fagales: Fagaceae), were manually damaged in an attempt to investigate their wounding response. Leaves were damaged with a hole punch early in the growing season to simulate herbivory. To another group of trees, the hormone, jasmonic acid, was applied to leaves *in situ* to determine if this volatile compound associated with wounding could elicit a defensive response in non-damaged trees. Foliar tannin concentration increased in both control and treated trees within 2 weeks after the experiment had been begun. Damaged trees exhibited a 56.5% increase in foliar tannin concentration and trees applied with jasmonic acid showed a 62.4% increase while control trees had only a 35.9% increase; however, these differences were not significant. Interestingly, manually damaged leaves demonstrated a 72.5% increase in crude tannin levels, whereas non-damaged leaves on damaged trees showed only a 42.3% increase. These differences approached significance and suggest that damage to leaves induced an increase in tannins, but this increase was not systemic. Moreover, damaged and jasmonate-treated trees had significantly fewer insects compared with control trees. Manually damaged leaves had the highest tannin concentration after treatment, and this was interpreted to be an induced defense or wounding response. Jasmonic acid appeared to have no effect on foliar tannin concentration; however, the number of herbivorous insects on jasmonic acid treated trees was similar to the number on damaged trees.

Key Words: induced defense, tannins, jasmonic acid, herbivory, *Quercus laevis*

### RESUMEN

Tejido foliar de los árboles del roble pavo, *Quercus laevis* Walter (Fagales: Fagaceae), fue dañado manualmente en un intento de investigar su respuesta a la herida. Las hojas fueron dañadas por una perforadora temprano en la temporada de crecimiento para simular herbivoría. A otro grupo de árboles la hormona ácido jasmónico fue aplicado a las hojas *in situ* para determinar si este compuesto volátil asociado con heridas podría obtener una respuesta de defensa en los árboles que no fueron dañados. La concentración de tanino foliar en los árboles del grupo experimental y también los del grupo de control aumenta dos semanas después del experimento fue empezado. Los árboles dañados exhiben un incremento de 56,5% en la concentración de taninos foliar, y los que hubieron un aplicación de ácido jasmónico mostró un aumento de 62,4% mientras que los árboles del grupo de control sólo tuvieron un aumento de 35,9%; no obstante, estas diferencias no fueron significativas. Curiosamente, las hojas dañadas manualmente demostraron un aumento de 72,5% en niveles de taninos crudos mientras que las hojas sin daño de árboles dañados mostraron un aumento del 42,3%. Estas diferencias se acercaron importancia y sugieren que el daño en las hojas indujo un aumento de taninos, pero este aumento no fue sistemático. Además, árboles dañados y los que recibieron un tratamiento de ácido jasmónico tenían significativamente menos insectos en comparación con los árboles del grupo de control. Las hojas dañadas manualmente tuvieron la concentración más alta de tanino después del tratamiento que fue interpretado a ser una respuesta de defensa o herir a inducida. Ácido jasmónico no parecía tener ningún efecto sobre la concentración de tanino foliar; sin embargo, el número de insectos herbívoros fue similar a los árboles dañados.

Translation provided by the authors.

Because of the loss of biomass, insect herbivory is traditionally believed to exert a strong selective pressure on plant evolution (Darwin 1859). This negative selective pressure by phytophagous insects is believed to explain the insect-specific toxic effects of plant secondary compounds such

as the rotenoids and pyrethrins (Harborne 1993). However, mild herbivory may be beneficial to plants (Agrawal 1998), while copious herbivory is detrimental to their survival and fitness. Plants have evolved an array of physical and chemical defenses to combat herbivory. For instance, plants

are capable of producing and accumulating secondary metabolites in foliar tissue in response to physical damage; this is known as 'induced defense' or more generally as a wounding response and is common throughout the plant kingdom (Karban & Myers 1989). Although the exact mechanisms are poorly understood, recent studies found that jasmonic acid has been associated with plant tissue after physical damage (Koo et al. 2009). This hormone then activates the defense genes of plants, ultimately leading to the accumulation of secondary metabolites (Pauwels et al. 2008; Gundlach et al. 1992). These secondary metabolites then help plants defend themselves against both pathogens and herbivores (Levin 1976).

The related concept of 'talking trees', first proposed in the early 1980s (Rhoades 1980; Baldwin et al. 1983), states that plants damaged by herbivores warn neighboring plants of an impending attack. Interest in inter-plant communication decreased in the late 1980s because a mechanism had not been discovered (Shonle & Bergelson 1995). However, Farmer & Ryan (1990) described at least one mechanism in which jasmonic acid undergoes methylation to form the more volatile compound, methyl jasmonate which is emitted as a gas from damaged plant tissue and travels through the air to warn neighboring plants of pending danger. Similar to jasmonic acid, methyl jasmonate has been shown to activate the defense genes of many terrestrial plants (Gundlach et al. 1992). Since the discovery of a mechanism behind talking trees, it has been the subject of extensive research (Dicke 2009; Engelberth et al. 2004). Methyl jasmonate can be sensed by some insects. Phytophagous insects which are negatively affected by plant secondary metabolites will often avoid leaves emitting methyl jasmonate (Birkett et al. 2000; Thaler et al. 2001). However, predators and parasitoid wasps may be attracted to such plants (Thaler 1999; Bruinsma et al. 2008). Thus, this chemical is apparently capable of affecting organisms on multiple trophic levels.

We attempted to induce defensive responses within foliar tissue in the native scrub oak species, *Quercus laevis* Walter. We attempted to determine if wounded trees produced a detectable defensive response in neighboring non-damaged leaves within the same tree. Oaks are well known to produce phenol-based secondary metabolites, especially tannins, which accumulate in foliar tissue in response to physical damage such as herbivory (Feeny 1970). Tannins defend foliar tissue by non-specifically binding and precipitating water soluble protein, including nutritional proteins and digestive enzymes of herbivores, thus reducing the nutritional quality of plant tissue to herbivores (Bate-Smith & Swain 1962; Feeny 1969). Tannins have a complex chemical structure with

no single species being responsible for their defensive nature (Mueller-Harvey 2001). Crude foliar tannin concentration was used to quantify the effectiveness of the plant hormone jasmonic acid on turkey oak trees and the surrounding insect community *in situ*. It was hypothesized that both manually damaged trees and those treated with jasmonic acid would cause an increase in foliar tannin concentration similar to simulated herbivore damage compared with non-damaged controls. The effect of these treatments on overall insect herbivory was also compared between treatment and control trees.

#### MATERIALS AND METHODS

Just after leaf flush in late spring of 2009, fifteen young (3-3.5 m tall) turkey oak (*Quercus laevis*) trees were haphazardly selected from an upland pine forest in the ecological preserve on the campus of the University of North Florida (Jacksonville, Florida; 30.271°N 81.514°W). To control for size differences among trees, the size and number of leaves of each tree were recorded. Prior to establishing the experimental treatments, the height of each tree was measured from its base at the soil surface to its highest point (cm); in addition diameter at breast height (DBH) (cm) and the total number of leaves for each tree were recorded. Trees were randomly assigned to one of 3 treatment groups with 5 replicates per group: 'damaged', 'jasmonate', or 'control'. Damaged trees received 10 holes on every other leaf made with a 5-mm hole puncher. Jasmonate trees had all leaves sprayed with 1.2 mM ( $\pm$ ) jasmonic acid (Sigma, St. Louis, Missouri, USA) dissolved in 1:10 ethanol: water solvent. Control trees had all leaves sprayed with the 1:10 ethanol: water solution only.

Because we chose young trees with relatively few leaves (see Table 1), we limited our leaf collection to 2 leaves per tree during each sampling period to minimize damage. Leaves were collected from all trees for chemical analysis before treatments were imposed. Two weeks post-treatment, leaves were again collected from all trees. For damaged trees, 1 damaged and 1 non-damaged leaf were collected at each date. After collection,

TABLE 1. TREE DBH, HEIGHT, AND NUMBER OF LEAVES BEFORE TREATMENT (MEAN  $\pm$  SEM). NO SIGNIFICANT DIFFERENCES EXISTED BETWEEN GROUPS BEFORE TREATMENT.

Treatment	DBH (cm)	Height (cm)	Number of Leaves
Control	13.6 $\pm$ 0.5	327.2 $\pm$ 10.4	198 $\pm$ 18
Jasmonate	15.3 $\pm$ 1.0	321.2 $\pm$ 27.7	249 $\pm$ 40
Damaged	13.4 $\pm$ 0.9	349.0 $\pm$ 22.1	230 $\pm$ 12

leaves were oven-dried at 60°C for 72 h and ground to a fine powder in a Wiley mill. Samples were stored in a desiccator at room temperature until further analysis. Total crude foliar phenol concentration was determined with a protein-binding radial diffusion assay developed by Hagerman (1986). Bovine serum albumin (BSA) protein (Sigma, St. Louis, Missouri, USA) was dissolved in an agar medium. Phenols, such as tannins, diffuse through the medium and cross-link with BSA which causes a bright white precipitation ring to form. Tannins were extracted by combining 50-mg aliquots of ground leaf tissue with 250  $\mu$ L of a 70% aqueous acetone solution (v:v) and periodically vortexed for 1 h. Samples were subsequently centrifuged at 14,500 g for 15 min. The supernatant was collected and used for tannin analysis. Ten  $\mu$ L of the collected extract were placed into sample wells and incubated at 30°C for 72 h. The diameter of the precipitation ring was measured with digital calipers (accurate to 0.01 mm). The average ring diameter (in  $\text{cm}^2$ ) was compared with a standard curve created with a commercially available tannic acid standard (ICN Biomedicals, Aurora, Illinois, USA). Foliar tannin concentration for each tree was calculated by averaging the tannin concentration from individual leaves and expressed as a percent of dry leaf mass.

The change in percent foliar tannin for individual trees was calculated by taking the percent foliar tannin post-treatment minus the percent of foliar tannin before treatment. Changes in tannin concentration were subsequently analyzed by a one-way ANOVA. In addition, within damaged trees both the post-treatment percent foliar tannin and the change in percent foliar tannin for damaged vs. non-damaged leaves were compared by a two-tailed independent samples *t*-test to determine if tannin levels increase systemically or are localized to damaged leaves. At 6 weeks post-treatment, 20 leaves from each tree were haphazardly selected and counted for the number of herbivorous insects. The number of leaf eating insects per 20 leaves was analyzed by a one-way ANOVA and the data were natural-log transformed before analysis to meet the homogeneity of variance assumption of ANOVA. A Tukey's HSD was used for post-hoc comparison of means after a significant effect during ANOVA.

## RESULTS

Prior to establishing experimental treatments, no significant difference existed between the 3 treatment groups in the following measurements: diameter at breast height (DBH) ( $F = 1.491$ ,  $df = 2, 12$ ,  $P = 0.264$ ), tree height ( $F = 0.417$ ,  $df = 2, 12$ ,  $P = 0.635$ ), and total number of leaves ( $F = 0.946$ ,  $df = 2, 12$ ,  $P = 0.415$ ) (Table 1). Thus, the treatment and control groups should not exhibit any size

bias. For all trees (both control and treatment groups) crude tannin levels increased from 3.1-3.6% (May 7) to 5.1-5.8% (May 21) dry mass (Fig. 1). While the change in percent tannin between groups was consistently greater for damaged (56.5% increase) and jasmonate trees (62.4% increase) compared with controls (35.9% increase), these differences were not statistically significant ( $F_{2,12} = 1.611$ ;  $P = 0.240$ ). However, levels of herbivorous insects were significantly lower on both damaged and jasmonate trees compared with control trees ( $F_{2,12} = 8.655$ ;  $P = 0.005$ ). Herbivore load was 45% lower on jasmonate-treated trees and 53% lower on manually-damaged trees compared with control trees (Fig. 2). Interestingly, within damaged trees, tannin levels were marginally higher on manually-damaged leaves compared with non-damaged leaves on damaged trees ( $t = 1.881$ ;  $P = 0.097$ ). Over the course of the study, tannin levels in damaged leaves increased twice as much as those from non-damaged leaves (3.6% vs. 1.8%). The differences between damaged and non-damaged leaves from the "damaged tree treatment" probably accounted for the original non-significant ANOVA and suggest that tannins do not increase systemically in response to wounding (Fig. 3).

## DISCUSSION

All trees exhibited a similar increase in foliar tannins following treatment but manually damaged leaves exhibited the greatest increase over

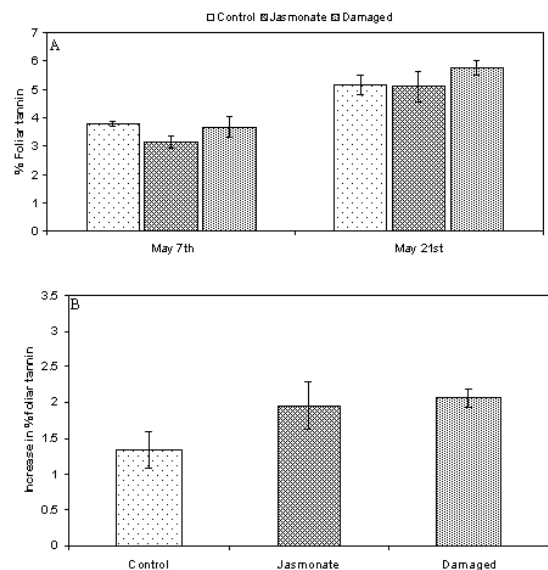


Fig. 1. (A) Percent foliar tannin for all groups immediately before and 2 weeks after treatment (mean  $\pm$  SEM). (B) Increase in percent foliar tannins after treatment (mean  $\pm$  SEM).

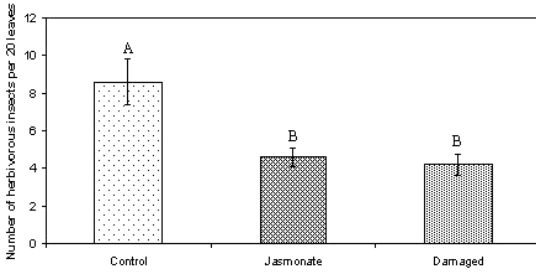


Fig. 2. The number of herbivorous insects found on 20 haphazardly chosen leaves 6 weeks after treatment (mean  $\pm$  SEM).

the course of the study period. However, significant effects in foliar tannins for damaged leaves may have been masked by seasonal trends and high inter-tree variation, but effects are similar to results from previous studies (Rossi et al. 2004; Wold & Marquis 1997). While the increase in crude foliar tannins partially reflects the seasonal trend for increased tannin production in *Q. laevis* (A. M. Rossi, unpublished data), non-damaged leaves adjacent to manually damaged leaves exhibited an increase in foliar tannins similar to control leaves, suggesting that damaged leaves accumulate phenolics at a faster rate than surrounding non-damaged leaves. Jasmonate-treated trees showed an increase in foliar tannin concentration similar to control leaves which suggested that the application of jasmonic acid had

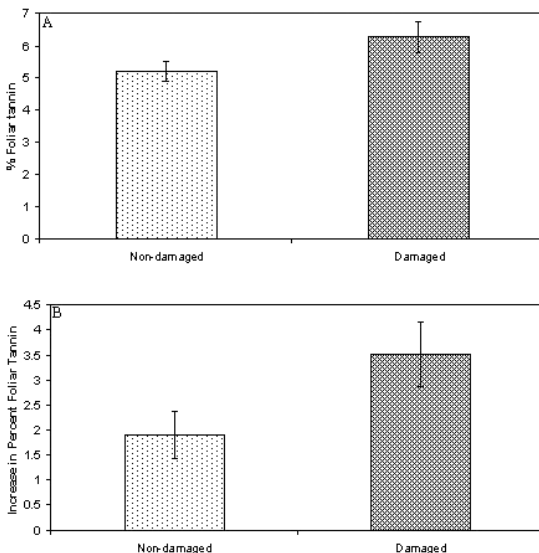


Fig. 3. (A) Percent foliar tannin for both non-damaged and manually damaged leaves on damaged trees after treatment (mean  $\pm$  SEM) on May 21. (B) Increase in percent foliar tannins between both non-damaged and damaged leaves on damaged trees (mean  $\pm$  SEM).

no effect on foliar tannin concentration. These data suggest that the wounding response appears to be localized to damaged leaves rather than systemic.

Many plant species are known to respond to jasmonic acid by the accumulation of secondary metabolites; however, some species do not. Cooper & Rieske (2008) conducted a study similar to ours in which they applied jasmonic acid to 2 species of chestnut trees. The treatment elicited the production of tannins in 1 species but not the other. In another study, the application of jasmonic acid did not elicit the accumulation of tannins within turtlegrass (*Thalassia testudinum*) (Arnold et al. 2008). *Quercus laevis* appears to be a species which does not accumulate tannins in response to jasmonic acid; at least at the levels used in this study.

Both jasmonate and damaged trees had fewer foliar insects compared with control trees. The accumulation of tannins may have protected damaged leaves from herbivores but jasmonate trees did not show a similar increase in foliar tannin concentration in response to treatment. This suggests that herbivores possibly sensed the jasmonic acid and chose to avoid jasmonate trees as indicated by other studies (Thaler et al. 2001; Bruinsma et al. 2008). For instance, Tschardt et al. (2001) correlated foliar damage with a decrease in insect diversity, although other studies conducted on the genus *Quercus* did not see such an effect (Hunter & Forkner 1999). Further research is needed to better understand how the defense response of *Q. laevis* affects the surrounding community.

Lastly, the crude tannin extract based on the Hagerman assay may lack enough precision to detect slight changes in phenolic levels. However, this assay is very useful because the precipitation ring produced is biologically relevant (since it is a measure of the protein binding ability of the extract). Although the proposed hypothesis was partially supported, the effects of jasmonic acid on *Q. laevis* remain inconclusive. For example, the application of jasmonic acid had no significant effect on foliar tannin concentration, but caused a significantly lower abundance of foliar insects. Perhaps a more controlled study (i.e., the use of greenhouse or laboratory studies) will eliminate variables enabling the subtle effects of jasmonic acid on the foliar tissue of *Q. laevis* to be understood. Future studies should focus on levels of natural enemies of herbivorous insects because parasitoids may detect subtle changes in the chemistry of damaged plants, resulting in higher rates of attack by natural enemies.

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