Effects of deer herbivory on chemical defenses of *Fagus grandifolia*

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Abstract

Plants exhibit a plethora of chemical responses to herbivory. Compared to studies on plant responses to insect herbivory, there are far fewer relating to mammalian herbivory. However, one well-known plant defense against mammalian browsing is the presence of tannins. Condensed tannins, as opposed to hydrolyzable tannins, are more well-studied. These polyphenols bind with protein to create an astringent taste that is repellent to animals. In general, phenolics are a diverse group of compounds involved with plant defense (Rehmen et al. 2012). Salicylic acid also plays a role in plant defense, particularly against pathogens (Ohse et al. 2016).

In an experiment by Ohse et al. in 2016, clipping of *Fagus sylvatica* (European beech) leaves and addition of *Capreolus capreolus* (roe deer) saliva caused an increase of leaf salicylic acid content. Condensed tannin content did not change in leaves or buds after application of the clipping and saliva treatment on those structures. This raises the question of whether tannin levels, as well as other defensive phenol levels, are inherent within the plant or change in response to herbivory.

Our study examined the potential long-term change in chemical composition of *Fagus grandifolia* as a result of exposure to herbivory by *Odocoileus virginianus* (white-tailed deer). We chose the deer and tree species present in the Northern Lower Peninsula of Michigan most similar to those used in the Ohse et al. study. In addition, there is no published research about the effects of deer herbivory on *Fagus grandifolia* leaves and buds. We hypothesized that chemical composition of leaves and buds between the deer exclosure plot and surrounding area would differ, with a significant increase in salicylic acid production in leaves and in general defensive compounds. We examined the relationship between years since browsing and the amount of chemical compounds present. Trees that experienced more recent browsing were hypothesized to have higher levels of defensive compounds.

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Signed,

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Materials and Methods

Collection of samples took place at two areas, a deer exclosure in the 1998 control plot of the UMBS Burn Chronosequence and the surrounding forest area. We selected 3 trees from each cardinal direction both inside and outside the exclosure. For the outside area, we limited the area to be selected from to 10 m from the exclosure fence. We took samples of 3 leaves per tree *F. grandifolia*, and several buds per tree. Samples were stored in Falcon tubes and stored on ice immediately after collection to minimize change in volatile substances. We also measured height of the tree, and took an average of 3 branches to determine how many years have passed since the tree was browsed. This method was taught to us by Jill Witt, and implemented counting of bud scars from the tip of a branch to the corresponding browsed portion, although we only completed this method for the trees outside the deer herbivory plot. We found years since browsing by counting the terminal bud scars, which gave a five year range for tracking growth (Waller et al. "Twig Age Sampling Protocol").

After freezing our samples, we used a milling machine to grind the leaf samples into a fine powder. We then weighed a portion of each sample ranging between 0.0200 g and 0.0300 g to use for total and simple tannin analysis.

Using the chemical analyses from our samples, we conducted t-tests comparing the compositions of different compounds present in *F. grandifolia* samples from inside and outside the exclosure. We then ran a regression analysis comparing the content of different compounds outside the exclosure against the number of years since the sample was browsed. The packages we used include 'ggplot', to show a plot of our distributions and variable relationships; 'scale', to modify data into scientific notation; and 'dpyr', to manipulate specific data parameters. A PCA model was used to find correlations between samples, years since browsed, and various chemical compounds.

Results

Because we found no significant results related to tannins in buds or leaves, we modified our analyses to examine the most significant results in the available data.

All or almost all leaf and bud samples (each 24 total) contained ergosterol acetate, while no leaf or bud samples contained salicylic acid. All leaf samples but only 1 bud sample contained α-tocopherol. The number of leaf and bud samples containing catechol were more similar, at 13 and 17, respectively (Table 1).

We found a statistically significant increase within the leaf samples for the frequencies (ions min^{-1}) of catechol and α-tocopherol inside of the deer exclosure compared to the outside (catechol, t-test, $P = 0.005$; α-tocopherol, Wilcoxon test, $P = 0.0242$; Fig.4, 5). We ran a linear regression using the average number of years since a sampled plant was browsed against the frequencies of phenols, but our group found no significant correlations between the catechol or α-tocopherol (catechol, $R^2 = 0.147$, $P = 0.120$; α-tocopherol, $R^2 = -0.077$, $P = 0.655$; Fig. 6).

For buds, we compared the frequencies of ergosterol acetate inside and outside of the exclosure and found a near statistically significant result, with higher frequencies inside the plot ($P = 0.060$; Fig. 1). Our group also found no statistically significant correlation between years since last browsed and the frequency of ergosterol acetate or phenols (ergosterol acetate, $R^2 = 0.109$, $P = 0.157$; catechol, $R^2 = 0.100$, $P = 0.167$; α-tocopherol, $R^2 = 0.029$, $P = 0.288$; Fig. 2, 3).

Discussion

Leaves/Buds: α-Tocopherol is a phenol that is released under stress (Munné-Bosch 2005). More leaf than bud samples contained α-tocopherol, which may result from leaves being more frequent targets of herbivory than buds. However, more research is required to support this idea. It is important to note that the buds were collected during the summer, which means that their chemical composition may have differed from that of fully developed buds. One of the main focuses of our study was salicylic acid content, which we expected to increase in leaves with exposure to herbivory. However, no traces of salicylic acid were found in either leaves or buds of *F. grandifolia* (Table 1). This may be because *F. grandifolia* naturally does not contain salicylic acid, instead using other chemicals for defense.

Inside/Outside: Catechol is a phenol that, upon activation, breaks down into toxins that negatively affect the growth and survival of insect herbivores (Mondal et al. 2017; Boeckler et al. 2016). The significantly higher leaf catechol content found inside the exclosure than outside suggests that *F. grandifolia* within the exclosure is defending itself against more vigorous herbivory by insects (Fig. 5). It is possible that the absence of deer, another major herbivore, has created an ideal environment for insect feeding. However, more research is required to explore these hypotheses. There was also significantly higher α-tocopherol content in leaves from inside the exclosure than outside (Fig. 4), which is consistent with elevated stress levels, catechol levels, and insect herbivory, though the connections between these have not been studied sufficiently to make conclusions. There was significantly higher ergosterol acetate content in buds from outside the exclosures than from inside. Ergosterol is found in the cell membranes of filamentous fungi and is often used as an indicator of fungal biomass (Ng et al. 2007), so this result indicates possible higher fungal biomass in outside buds (Fig. 1). It can be speculated that deer herbivory increases the likelihood of fungal growth in buds, possibly due to salival traces and leaf damage. More studies would need to be done to properly explore this topic.

Years since browsed: When we made a linear regression comparing leaf catechol and α-tocopherol with years since browsed, another unexpected (though statistically insignificant) trend was observed (Fig. 6). There were lower phenol (catechol and α-tocopherol) levels in leaves of trees that experienced more recent browsing. Based upon our observations during data collection, lower defense compounds outside of the deer exclosure may be due to lower overall plant health. The *F. grandifolia* outside the exclosure were observed to be highly browsed and therefore under high stress, which may be a confounding variable in our study. Ergosterol acetate and phenol content in buds both increase as browsing becomes more recent (Fig. 2, 3). This is consistent with the aforementioned hypothesis relating deer herbivory and fungal growth, but not with our predictions concerning phenols and other phytohormones. Contrasting the study by Ohse et al., our finding suggests that phytohormone production in *F. grandifolia* can change in response to environmental input, though we found no conclusive results concerning herbivory (2016).

Tables and Figures

Table 1. Number of *Fagus grandifolia* leaf and bud samples found to contain α-tocopherols, catechol, ergosterol acetate, and salicylic acid. There were a total of 24 samples each for leaves and buds.

Compound	Leaf Samples	Bud Samples
α-Tocopherol	24	1
Catechol	13	17
Ergosterol Acetate	24	23
Salicylic Acid	0	0

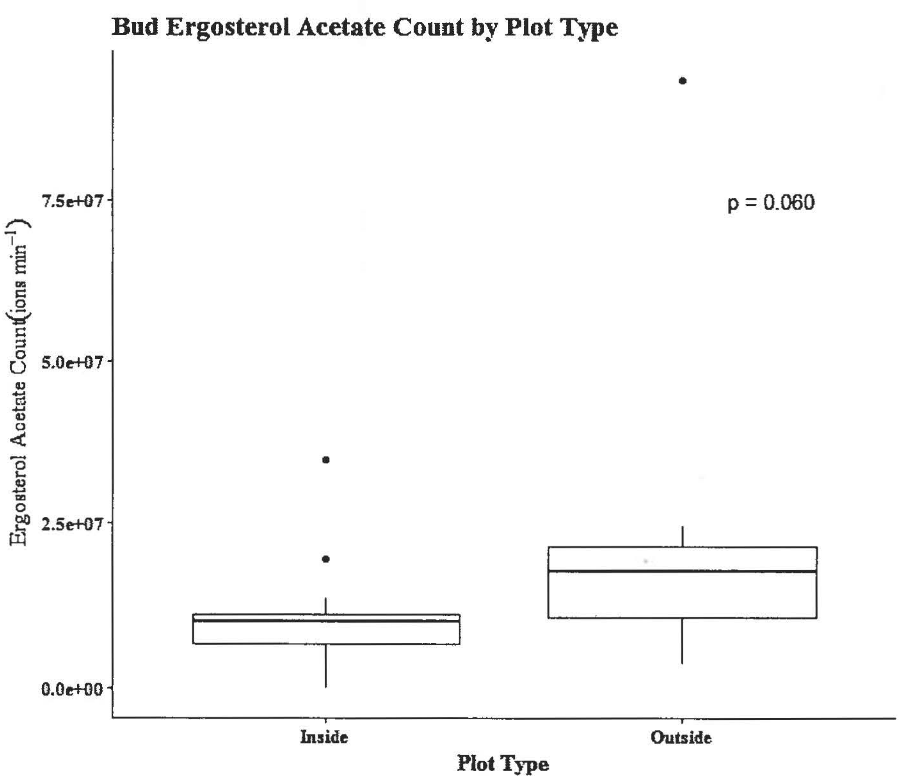

Figure 1. Ergosterol acetate frequencies in buds inside and outside the exclosure plots.

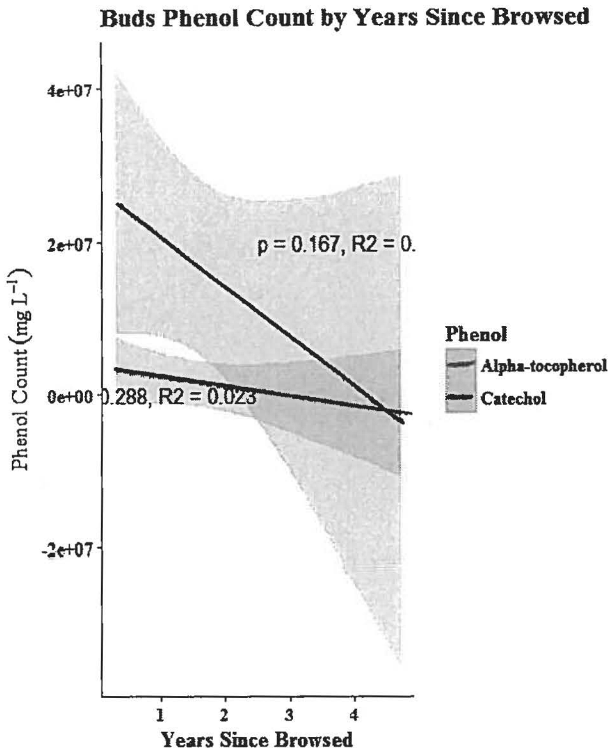

Figure 2. Phenol concentration of α-tocopherol and catechol in buds against years since browsed outside of the exclosure plot.

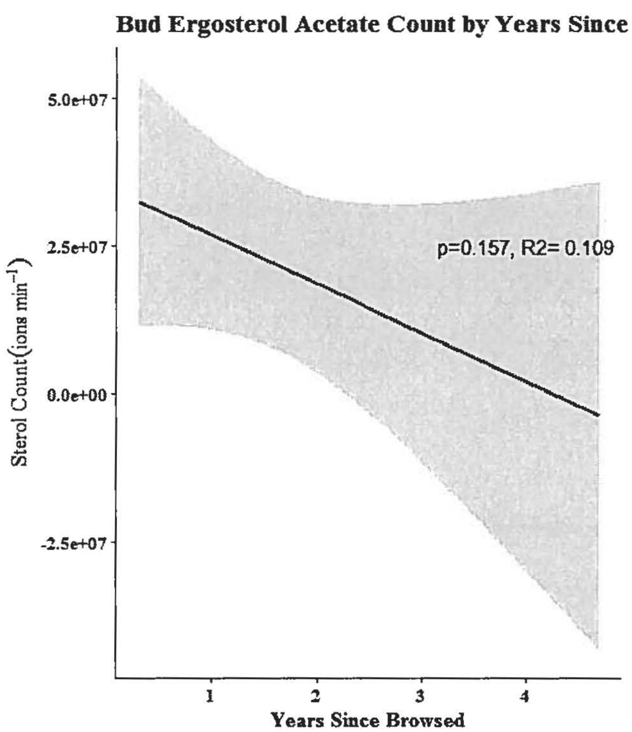

Figure 3. Ergosterol acetate frequency in buds against years since browsed outside the exclosure plot.

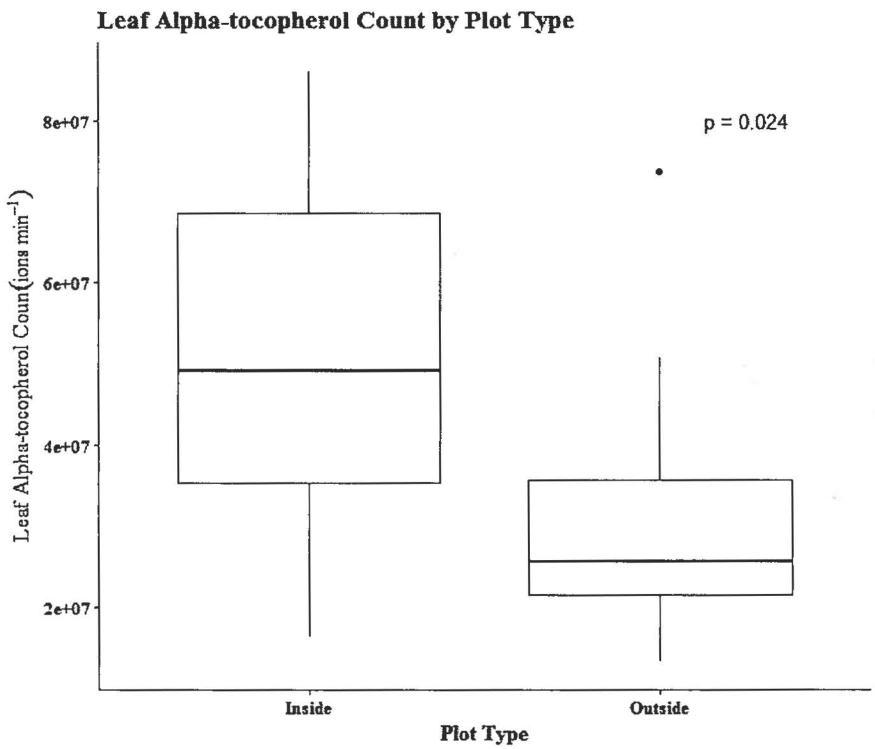

Figure 4. α-tocopherol frequency in leaves inside and outside the exclosure plot.

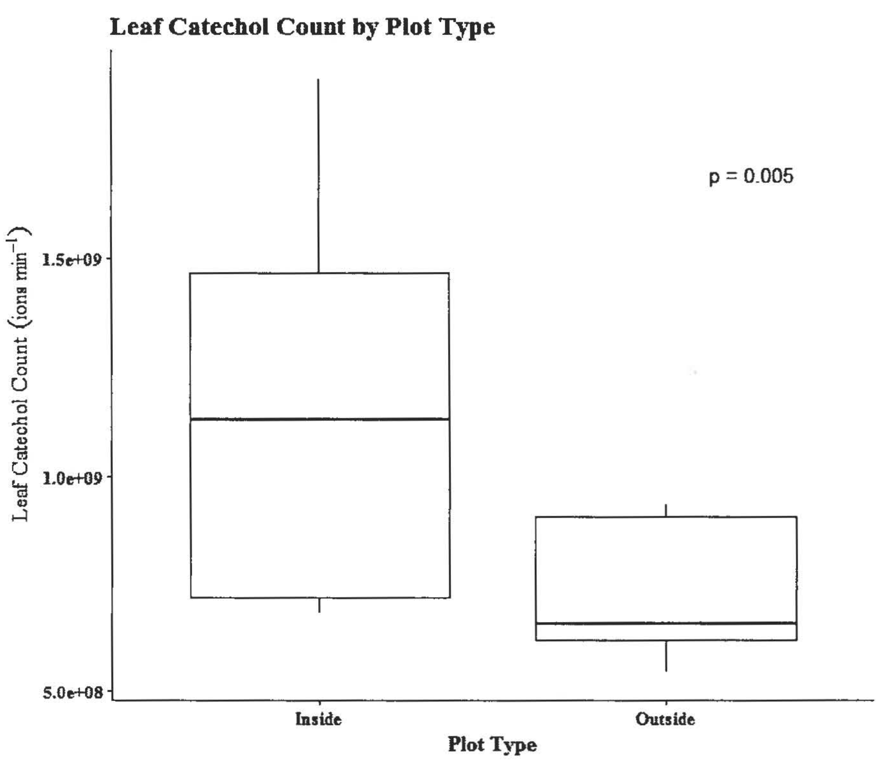

Figure 5. Catechol and α-tocopherol frequency in leaves inside and outside the exclosure plot.

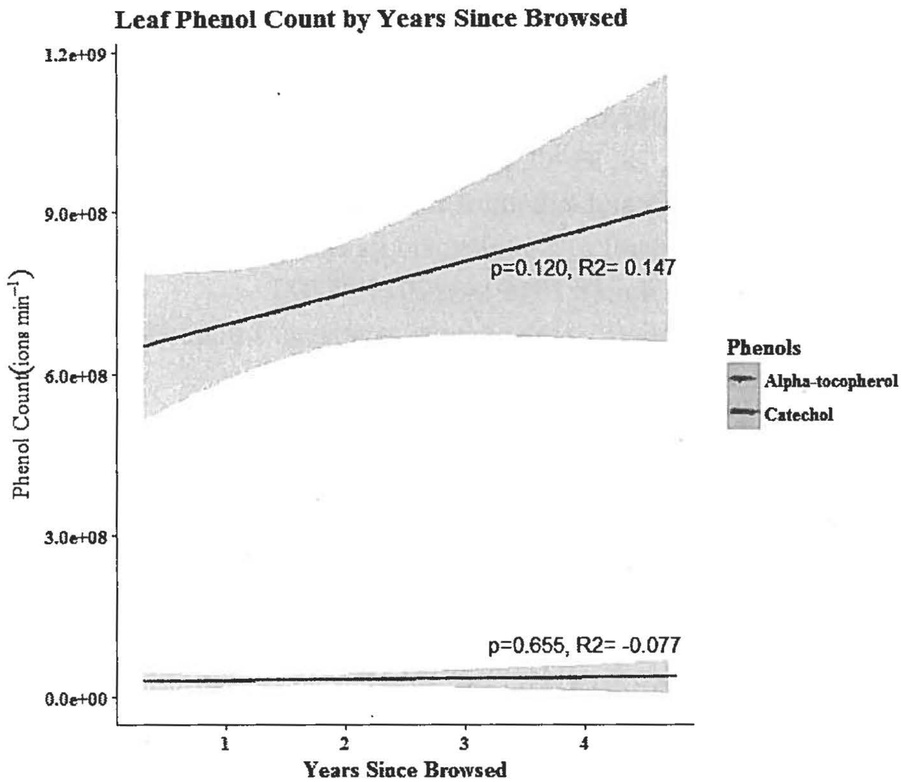

Figure 6. Phenol frequency of α-tocopherol and catechol in leaves outside the exclosure plot.

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