

PRELIMINARY ANALYSIS OF FREQUENCY AND COMPOSITION OF TANNINS IN ACORNS, WEEVILS, AND FRASS

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Abstract

Curculio sp. weevils develop through several instars living in and consuming acorns, depositing frass within the acorn as they feed and grow. These weevils live on this diet despite the presence of tannins, reportedly higher in red oaks than white oaks. We examined the amount and composition of tannins in acorns (*Quercus macrocarpa* – a white oak and *Q. rubra* – a red oak), weevils and frass by subjecting them to liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS). By comparing the mass spectroscopy data consisting of molecular weights and fragmentation data, we can determine if the tannins in acorns provide any nutritional value to the weevil. Tannins that weevils ingest are affected differently; some pass through without being utilized, appearing in the frass. Others are metabolized and some of the breakdown products are absorbed by the weevil gut. The tannins we have found in the acorns, weevils, and frass are from two classes of tannins: ellagic acid and gallic acid. The ellagic acid type tannins are seen at a higher frequency than gallic acid tannins. Most of the tannins found in acorns were also found in frass, but very few tannins were found in larvae tissue.

Introduction

Oaks (*Quercus* sp.) possess a wide variety of tannins, often in high concentrations, in their wood, leaves, and acorns. Condensed and hydrolyzable tannins are distinct both chemically and structurally. These secondary metabolites may be produced to defend the plant from herbivores (Feeny 1970) or act as a qualitative defense against digestive cellulases and proteases produced by bacteria and fungi (Zucker 1983). If tannins are indeed a herbivore defense mechanism, then weevil (*Curculio* sp.) larva that grow and develop in acorns (Gribko et al. 2002) have either developed a tolerance for high tannin concentrations or they metabolize the tannins. Some tannins (e. g. – digalloyl-hexahydroxydiphenyl-glucose) have a glucose that could be made available by metabolism of the molecule. What is the fate of the tannins as acorn endosperm is digested by the weevil larva?

Cantos et al. (2003) and Meyers et al. (2006) have identified some of the common condensed and hydrolyzable tannins from European *Quercus* sp. and a related California oak species (*Lithocarpus densiflorus*). However, most of the North American *Quercus* species have yet to be described with respect to the tannins they produce. Furthermore, tannins could be used for further phylogenetic analysis of the genus *Quercus*.

Methods

SAMPLE PREPARATION

Acorns were harvested from trees in the Kansas City area before they dropped in fall 2006. The acorn samples were dried in an oven for seven days at 37°C and ground to a fine powder prior to extraction. Weevil larva and frass were treated without being subjected to the drying process. All samples were treated with a solution of 80% MeOH/H₂O with 0.8mM NaF at a ratio of 0.1 g of sample to 1 ml of solution. The weevil larva were pulverized in the extraction solution to free the tannins from the tissues. To ensure that the solution was completely extracted, the samples were shaken for an hour. A crude filtration was conducted on each of the samples utilizing a cotton plugged pipette. Following the crude filtration, the solution was passed through a syringe filter with a 0.2 µm nylon membrane to remove additional solids. The filtered samples were subjected to liquid chromatography electrospray ionization mass spectrometry (LC/ESI/MS). The LC/ESI/MS separates the tannin mixture on a reverse-phase column prior to introduction into the mass spectrometer. During the separation process UV data is collected and the mass spectroscopy provides molecular weights and fragmentation data on the individual tannins.

INSTRUMENTATION – HPLC/DAD/ESI-MS/MS

LC/ESI/MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x 0.5 mm i.d., Zorbax XDB – C18 3.5 µm (Agilent, Palo Alto, CA). Flow rate was 5.00 µL/min, injection volume was 0.5µL, and column temperature was 25 °C. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N₂), 5.00 L/min; dry temperature, 325 °C; trap drive, 78.0; skim 1, -40 V; lens 1, 5.00 V; octopole RF amplitude, 200.0 Vpp; capillary exit, -200 V. The ion trap mass spectrometer was operated in negative ion mode scanning from m/z 50 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 70000 units and maximal accumulation time was 200000 µs. MS-MS was operated at a fragmentation amplitude of 1.0 V, and threshold ABS was 20,000 units.

LIQUID CHROMATOGRAPHIC SEPARATION

The constituents were separated using a water (A) and methanol (B) gradient (each containing 0.1% formic acid). Initial conditions were 3% methanol increasing to 25% methanol at 6 minutes increasing to 35% at 25 minutes increasing to 90 % at 35 minutes holding at 90% to 40 minutes and returning to starting conditions at 45 minutes. The detection wavelength was 254nm (Cantos et al. 2003).

Table 1. Tannins found in *Quercus macrocarpa* L. acorns, weevils infesting the acorns, and frass.

Tannin and Molecular Wt.	Acorn	Weevil	Frass
ellagic acid 301 (A)*	present	present	present
valoneic acid dilactone 469 (A)	present	present	present
dihexahydroxydiphenyl 783 (B)**	present	absent	present
digalloyl-hexahydroxydiphenyl-glucose 785 (B)	present	absent	present

*A represents an ellagic acid type tannin (301 portion of valoneic acid dilactone molecule below)

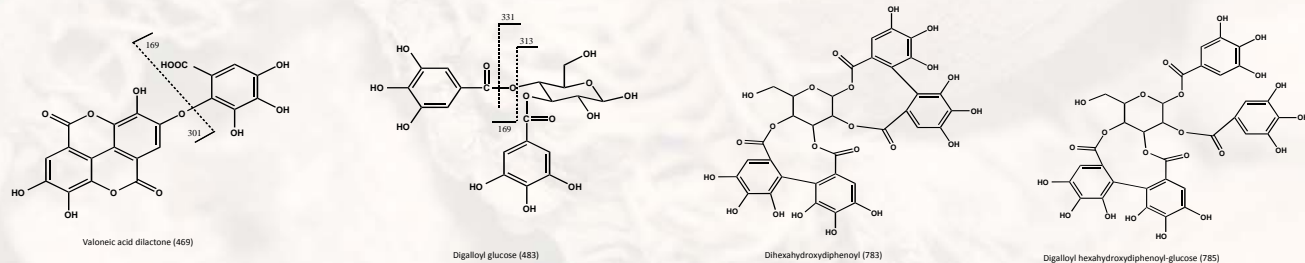
**B represents a gallic acid and HHDP tannin (169 portion of valoneic acid dilactone molecule below)

Table 2. Tannins found in *Quercus rubra* L. acorns, weevils infesting the acorns, and frass.

Tannin and Molecular Wt.	Acorn	Weevil	Frass
ellagic acid 301 (A)*	absent	absent	present
valoneic acid dilactone 469 (A)	present	present	present
digalloyl glucose 483 (A)	present	absent	present
dihexahydroxydiphenyl 783 (B)**	present	present	present
digalloyl-hexahydroxydiphenyl-glucose 785 (B)	present	absent	present

*A represents an ellagic acid type tannin (301 portion of valoneic acid dilactone molecule below)

**B represents a gallic acid and HHDP tannin (169 portion of valoneic acid dilactone molecule below)



Results

Of the five tannins investigated, four of them (ellagic acid, valoneic acid dilactone, dihexahydroxydiphenyl, and digalloyl-hexahydroxydiphenyl-glucose) were found in both *Q. macrocarpa* (Table 1) and *Q. rubra* (Table 2). Digalloyl-glucose was found in *Q. rubra*, but not in *Q. macrocarpa*. Ellagic acid and valoneic acid dilactone were found in *Q. macrocarpa* acorns, weevils, and frass (Table 1). Valoneic acid dilactone and digalloyl hexahydroxydiphenyl glucose were present in *Q. rubra* acorns, weevils, and frass (Table 2). Of the three materials analyzed, weevil larva tissue was most likely to lack tannins, specifically dihexahydroxydiphenyl and digalloyl-hexahydroxydiphenyl-glucose from *Q. macrocarpa* and digalloyl glucose and digalloyl-hexahydroxydiphenyl-glucose from *Q. rubra*.

In *Q. rubra*, ellagic acid (301 portion of valoneic acid dilactone molecule below) was present in frass, but not in either the acorn or weevil larva. Not only were fewer tannins present in the weevil larva than in acorns or frass (Tables 1 and 2), but the relative concentration was lower as well.

Discussion

A possible explanation for the appearance of ellagic acid in frass is that larger tannin(s) were metabolized, leaving this basic tannin building block. Perhaps the weevil larvae metabolize some tannins to acquire the glucose that is sometimes present.

Feeny (1970) found that tannins did not cross the peritrophic membrane of the winter moth (*Operophtera brumata*, Geometridae - Lepidoptera) midgut. We interpret our data to mean that the same is true of *Curculio* sp. larvae, that the tannins we did find in the larvae were from acorn endosperm in the gut. However, we did not dissect the larvae gut to determine where in the larvae body the tannins were located. This will be addressed in further research.

Despite the perceived differences between white and red oaks, we saw more chemical similarities than differences. Based on this very limited comparison between white and red oaks, our results suggest that the chemical differences between these two *Quercus* species is more quantitative than qualitative.

Literature Cited

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