Seed dormancy detection by eastern gray squirrels (*Sciurus carolinensis*)

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ABSTRACT

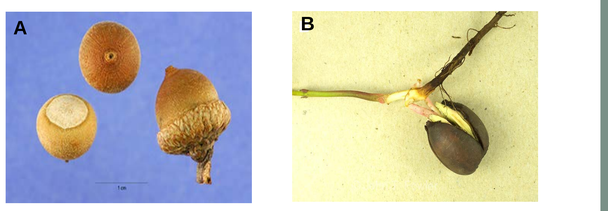
Eastern gray squirrels (*Sciurus carolinensis*) serve as seed dispersers by caching seeds in dispersed sites for later consumption. Specifically, squirrels cache dormant seeds whereas germinating and stratified seeds are consumed immediately. We tested the sensitivity of squirrels to different physical and chemical cues of dormancy. Gas chromatography-Mass spectrometry indicated that dormant red oak (*Quercus rubra*) seeds have a wax layer covering the shell, and Scanning Electron Microscopy analysis suggested that the wax layer is lost as seeds break dormancy and germinate. This breakdown leads to the release of possible germination and cell metabolism byproducts (e.g. acetaldehyde) and seed-kernel compounds (e.g. pyrogallol). To determine the characteristics that squirrels use to identify dormancy in seeds, we evaluated squirrel sensitivity to: 1. loss of shell wax simulated by scraping wax off the pericarp using sandpaper; 2. byproducts from degradation of kernel and shell wax compounds simulated by spraying dormant red oak seeds with 40ppb acetaldehyde solution; and 3. release of kernel compounds to shell simulated by spraying 0.02% pyrogallol solution on dormant red oak seeds. We paired these treatments with control red oak seeds sprayed with distilled water. We presented 20 seeds in each of the 4 categories to squirrels on Purdue campus. We found that scraped seeds and seeds treated with acetaldehyde were marginally more likely to be consumed by squirrels in comparison to control seeds (P=0.09). Additionally, squirrels travelled for a shorter time before caching scraped seeds (P=0.06) and spent longer quantities of time exhibiting paw manipulation behaviors with acetaldehyde treated seeds (P=0.03). The lower caching time investment associated with scraped seeds suggests that squirrels perceive seeds without wax coating to be of lower value. Increased paw manipulation behaviors with acetaldehyde treated seeds suggests that squirrels utilized taste receptors to detect acetaldehyde. Pyrogallol treatment was never a significant predictor of squirrel behaviors. From these results, we concluded that squirrels use 2 cues to detect lack of dormancy in seeds: the physical loss of wax from pericarps and the release of chemical byproducts from wax degradation (i.e. acetaldehyde).

INTRODUCTION & HYPOTHESES

Rodents can detect dormancy status of seeds using physical and chemical cues. In this study, we tested 3 possible cues used by eastern gray squirrels (*Sciurus carolinensis*) to detect dormancy of red oak (*Quercus rubra*) acorns:

1. Loss of wax from shell (simulated by scraping)
2. Aromatic by-products from wax degradation (spraying acetaldehyde on seed)
3. Escape of pyrogallol from kernel to shell (spraying pyrogallol on seed)

FIGURE 1. Seed types. Photographs of dormant Northern red oak seeds or *Q. rubra* (A) and germinating red oak (B)



LABORATORY & DATA ANALYSIS METHODS

Gas chromatography-Mass Spectrometry (GC-MS)

1.Shells and kernels washed separately in methanol, acetone, hexane
2.Washings analyzed by GC-MS
3.Repeated after germinating seeds in germination chamber
4.Three replicates performed
5.Total of 3 replicates x 2 seed portions (shell and kernel) x 2 dormancy status (germinating and dormant) x 3 solvents = 36 GC-MS chromatograms
6.Compounds scored for 36 chromatograms by searching NIST standard reference database with Wsearch

Foraging trials

- Presented free-ranging eastern gray squirrels with a sequence of seed types:
  1. Control: dormant red oak soaked in distilled water for 5 mins (Figure 1)
  2. Scraped: surface of dormant red oak is scraped with sandpaper
  3. Pyrogallol: dormant red oak sprayed with 0.02% pyrogallol for 5 mins
  4. Acetaldehyde: dormant red oak sprayed with 40ppb acetaldehyde for 5 mins
- Video recorded squirrels handling seeds and recorded
  1. Seed fate (cached = 1 or not cached =0)
  2. Travel time (minutes)
  3. Time spent in paw manipulation behaviors (minutes)
  4. Number of head flicks
- Regressions of behavior against seed weight, temperature, treatment type

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RESULTS

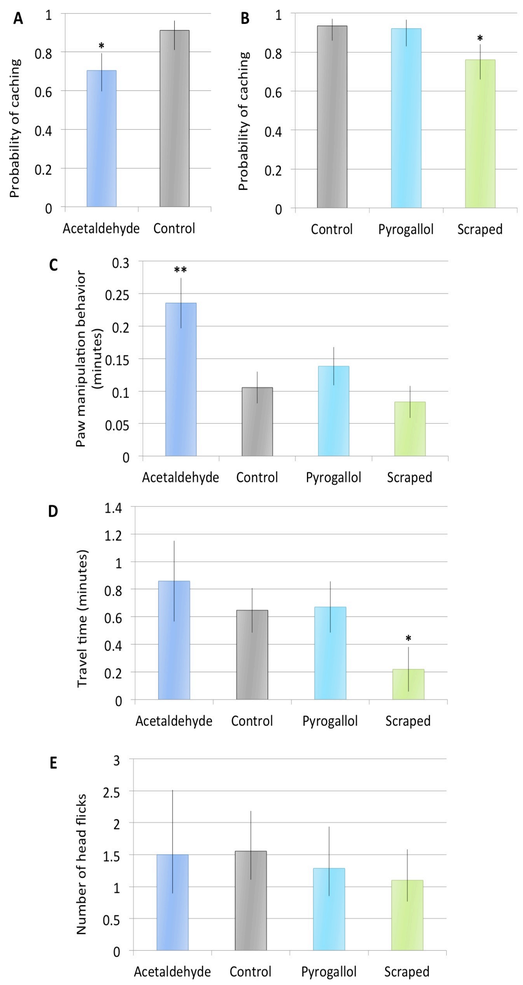
TABLE 1. Classes of chemical compounds occurring on shells of dormant and germinating red oaks (*Quercus rubra*). Chemicals detected using Gas chromatography-Mass spectrometry (GC-MS)

	Germinating	Dormant
Alcohols	1, 30 triacontanediol pyrogallol	1, 30 triacontanediol
Plant Hormones	Gamma sitosterol Stigmast 4-ene-3-one 24 methylene cycloarctanol Beta sitosterol	Beta sitosterol 24 methylene cycloarctanol Stigmastan 3,5 diene
Fatty acids	-	Linoleic acid Stearic acid Tricosanoic acid Tetracosanoic acid Hexadecanoic acid
Long chain fatty acid esters		Octadecyl ester Docosanoic acid- nonyl ester

TABLE 2. Classes of chemical compounds occurring in kernels of dormant and germinating red oaks (*Quercus rubra*). Chemicals detected using Gas chromatography-Mass spectrometry (GC-MS)

	Germinating	Dormant
Aldehydes	Furfural 2-furancarboxaldehyde	Furfural 2-furancarboxaldehyde
Alcohols	Pyrogallol 2,3 butanediol	Pyrogallol 2,3 butanediol
Plant hormones	Gamma tocopherol Gamma sitosterol	Gamma tocopherol Gamma sitosterol
Fatty acid esters	Hemipenic acid Hexadecanoic acid Linoleic acid	Hemipenic acid Hexadecanoic acid Linoleic acid
Ketones	3,5 dihydroxy 6 methyl 2,3 dihydro 4 H-pyran-4-one	3,5 dihydroxy 6 methyl 2,3 dihydro 4 H-pyran-4-one
Long chain fatty acid esters	Octadecyl ester	Octadecyl ester
Carbohydrate	6-deoxy-D-galactose	6-deoxy-D-galactose

FIGURE 2. Plots of probability of caching seeds (A and B), average paw manipulation (C), average travel time (D) and average number of head flicks (E) across treatment groups



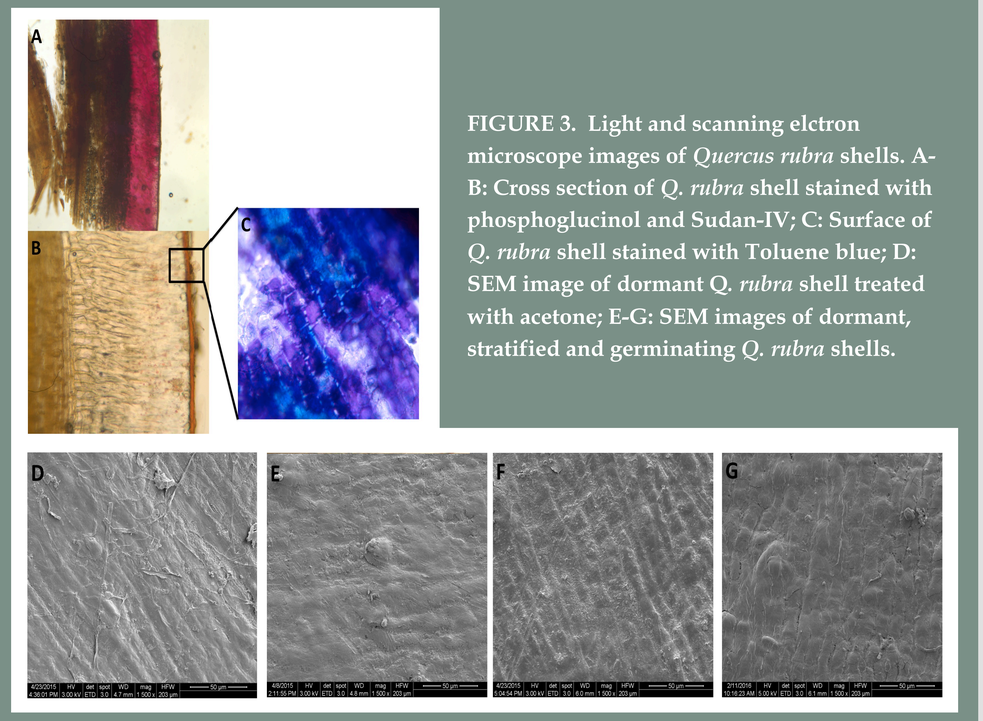


FIGURE 3. Light and scanning elctron microscope images of *Quercus rubra* shells. A-B: Cross section of *Q. rubra* shell stained with phosphoglucinol and Sudan-IV; C: Surface of *Q. rubra* shell stained with Toluene blue; D: SEM image of dormant Q. *rubra* shell treated with acetone; E-G: SEM images of dormant, stratified and germinating *Q. rubra* shells.

CONCLUSIONS

Squirrels travelled with scraped seeds for shorter time and shorter distances before caching, suggests seeds without wax are less valuable. Increased paw manipulation behaviors with acetaldehyde seeds suggests detection of acetaldehyde by taste. *S. carolinensis* detects dormancy using a combination of 2 cues:

1. Loss of wax in stratified red oak seeds (confirmed by SEM in Figure 3)
2. Chemical byproducts (e.g. Acetaldehyde) released from wax breakdown