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Source: Madroño, 67(4) : 165-169

Published By: California Botanical Society

URL: <https://doi.org/10.3120/0024-9637-67.4.165>

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LEAF HAIR TUFTS FUNCTION AS DOMATIA FOR MITES IN *QUERCUS AGRIFOLIA* (FAGACEAE)

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ABSTRACT

An identifying feature of *Quercus agrifolia* Née (Fagaceae) is the presence of hair tufts on lower leaf surfaces. In other plant species, hair tufts act as domatia for arthropods such as mites, which in turn feed on leaf fungi or small herbivores and possibly benefit plant health. However, this mutualistic relationship remains untested in *Q. agrifolia*. In this study two primary questions were addressed within a natural stand of *Q. agrifolia* in San Luis Obispo, CA: 1) Do hair tufts act as domatia for mites? and 2) Does the removal of hair tufts impact mite abundance, herbivory or fungal pathogens on leaves? In an observational study of 377 leaves from 20 trees, we found a significant association between the presence of hair tufts and the presence of mites. When we experimentally removed hair tufts, we found a significant reduction in mites, yet there was no impact on leaf herbivory or necrosis. We conclude leaf hair tufts on *Q. agrifolia* serve as domatia for mites, but we found no evidence that mites reduce herbivory or fungal pathogens. Thus, while mites likely benefit from housing provided by hair tufts on *Q. agrifolia*, it is unclear that the tree benefits from the mites, i.e., whether this is a mutually beneficial relationship.

Key Words: acrodomatia, coast live oak, commensalism, mite, mutualism, plant-animal interaction.

Leaf domatia are protective structures or ‘houses’ for arthropods and may represent a plant-arthropod mutualism. The arthropod benefits from protection provided by the host plant (e.g., Norton et al. 2001; Faraji et al. 2002; Grostal and O’Dowd 1994), while the host plant benefits from the arthropods’ active removal of herbivores or fungal pathogens (e.g., Agrawal and Karban 1997; Romero and Benson 2004; Norton et al. 2000; Weber et al. 2016). Across angiosperms, domatia have evolved independently hundreds of times and display a wide range of morphologies including tiny caves, pits, or hair tufts (O’Dowd and Willson 1989).

On the central coast of California, hair tufts on the abaxial leaf surface of *Quercus agrifolia* Née (coast live oak) are commonly observed (Fig. 1) and are a key feature used to distinguish this species from other California live oaks (Baldwin 2012). Previous work in the northern portion of the range of *Q. agrifolia* found that predatory and fungivorous mites (*Tydeus* sp.) occupied 34% of leaf hair tufts (Pemberton and

Turner 1989). However, it is unclear whether mites were more likely to occupy hair tufts than other areas of the leaf (i.e., whether hair tufts function as domatia) and, if so, whether mites benefit tree health by reducing leaf herbivores or fungal pathogens.

In this study we address three specific questions within a southern population of *Q. agrifolia*: Is there variation in the number of leaf hair tufts among individual trees? Are mites more likely to be found in hair tufts as opposed to other areas of the leaf? And finally, does the removal of hair tufts impact the number of mites, herbivory, and fungal damage?

METHODS

Study Design

This study took place in a natural stand of *Quercus agrifolia* in “Poly Canyon” at California Polytechnic State University, San Luis Obispo, California (latitude 35.3057, longitude –120.6581).

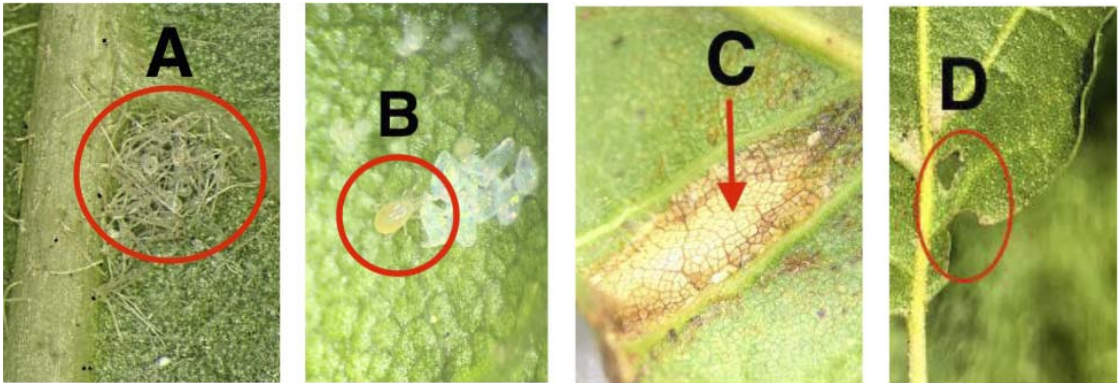


FIG. 1. *Quercus agrifolia* abaxial hair tufts (A), predatory mite in the family Phytoseiidae (B), necrosis due to fungal or bacterial pathogens (C), and herbivory (D)

To address our first study question, we conducted an observational study on 22 February 2019. Twenty teams, each comprised of two to four students, were assigned a random GPS coordinate within the study population and sampled leaves from the nearest tree. Random GPS coordinates were obtained by creating a roughly nine-acre polygon around oak woodland habitat in Poly Canyon using ArcGIS software (ArcGIS release 10.6.1, ESRI, Redlands, CA) and randomly sampling coordinates from within the polygon. Thirty leaves were haphazardly sampled, each from a unique branch within the sub-canopy. For each leaf, the petiole was cut using a razor blade to avoid flinging any mites off the leaf and leaves were placed individually in a plastic bag. Bags were carefully transported back to the lab in plastic trays so as to cause minimal disturbance to any mites.

In the lab, each team of students recorded data on 2–3 leaves from each of the 20 sample trees. This was done in order to avoid confounding the effects of student group and sample tree. For each leaf, the number of leaf vein axils with hair tufts was recorded. Hair tufts were defined as clusters of three or more hairs. For example, in Fig. 2, leaf secondary vein axils 3 and 6 contain hair tufts. To determine the association between hair tufts and mites, a single axil on each leaf was observed for the presence or absence of mites. All observations were made with a dissecting microscope at 10-40X magnification. Mite presence included larvae, nymph, or adult mites (eggs were excluded due to uncertainty in their identification). In order to sample approximately the same number of ‘no hair tuft’ as ‘hair tuft’ leaf axils for each tree, each leaf was pre-assigned to either the ‘no hair tuft’ or ‘hair tuft’ category. If ‘no hair tuft’ category was assigned, an axil without a hair tuft was selected for observation, always beginning with the second leaf vein axil from the bottom and to the right of the mid vein (Fig. 2, axil 2), and moving counterclockwise until an axil without hair tufts was located (in Fig. 2 this would be axil 2). Upon locating a suitable axil, the absence or presence of

mites was recorded. The same protocol was used for sampling leaves pre-assigned to the ‘hair tuft’ category (in Fig. 2 this would be axil 3).

Next, we performed a manipulative experiment to determine whether removal of hair tufts impacted

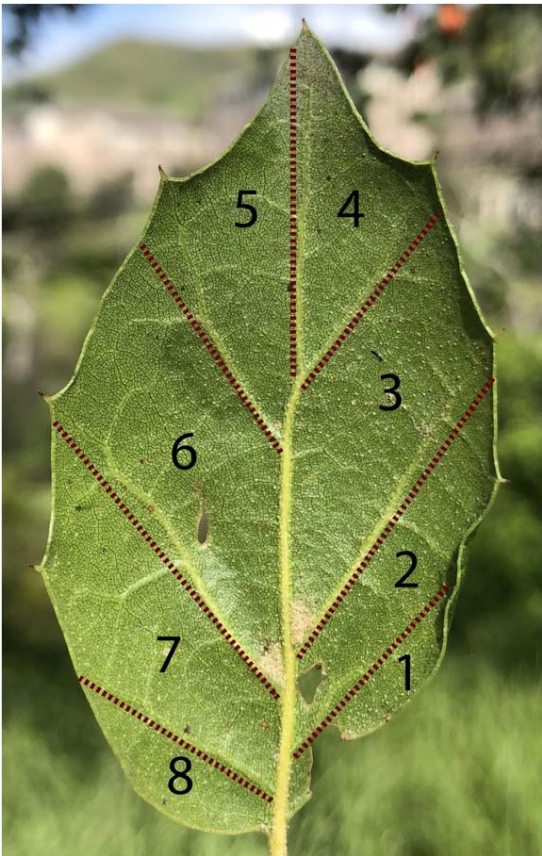


FIG. 2. Method of partitioning leaf surface for estimating necrosis and herbivory based on secondary leaf veins. Note hair tufts are present in vein axils 3 and 6.

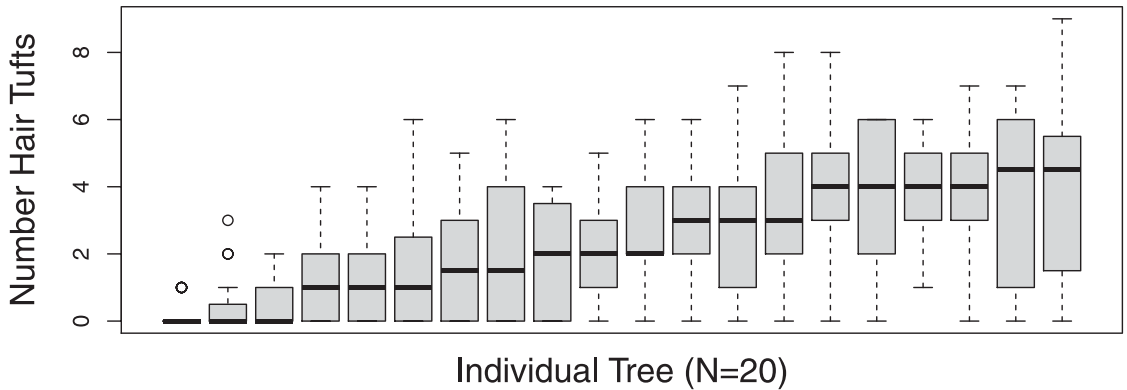


FIG. 3. Boxplot indicating variation in the number of hair tufts per leaf across *Quercus agrifolia* trees, ordered by median value. Number of leaves sampled per tree ranged from 18–24, for a total of 397 leaves.

the abundance of mites and leaf health. This experiment took place between 25 February through 10 May 2019, using a paired study design on 20 haphazardly sampled trees. Within each tree, 10 branches were selected that met the following criteria: branches contained at least 5 fully expanded leaves, were close enough to the ground to allow easy access by researchers, and were not in contact with any neighboring branches (to reduce the opportunity for mites to move from untreated to treated branches). Branches were then randomly assigned as removal or control treatment. On the ‘removal treatment’ branches, all hair tufts located in leaf vein axils on the abaxial surface were removed by gently scraping the leaf surface with a sharp tool (e.g. a scalpel). This was performed on every leaf within the branch. On ‘control treatment’ branches, abaxial leaf surfaces were scraped immediately adjacent to but just outside all leaf vein axils with hair tufts, thus leaving the hair tufts intact. The control treatment accounts for any effect of leaf scraping, for example, potential damage to the epidermis of the leaf.

Treatments were left untouched for 4–6 weeks, at which point we returned to assess mite abundance and leaf health. On each branch, we removed three leaves, always sampling the 3rd, 4th, and 5th fully expanded leaf from the apical meristem. The three leaves were placed in a plastic bag, transported back to the laboratory, and assessed within 24 hours of collection. Using a dissecting scope, on each leaf we recorded the number of leaf vein axils with: hair tufts, mites, herbivory, necrosis due to fungal or bacterial pathogens, and fly larvae/larvae scars (Fig. 1). Herbivory was defined as leaf areas with visible physical damage, while necrosis was defined as leaf areas with visible discoloration or decay. Fly larvae/larvae scars were not included in downstream analyses, as only 27 of 450 leaves had any presence of larvae/larvae scars.

Statistical Analyses

To assess whether there was phenotypic variation among trees in the number of hair tufts per leaf, we used a generalized linear model with a Poisson error distribution (base stats package in R (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). The response variable was ‘number of hair tufts’ while the predictor variable was tree identity ($n = 20$).

To determine whether there was an association between hair tufts and mites, we used a mixed effects logistic regression (*glmer* function in the *lme4* R package, Bates et al. 2015). Abaxial leaf vein type was included as a fixed effect (hair tufts vs. no hair tufts), while tree identity ($n = 20$) and observer group ($n = 20$) were included as random effects. The binary response variable was mite presence vs. absence.

To assess whether there was an effect of experimental treatment (control vs. hair tuft removal) on mite abundance and leaf health we used a mixed effects linear regression with a Poisson error distribution (*glmer* function in the *lme4* R package, Bates et al. 2015). Treatment was included as a fixed effect (control vs. hair tuft removal), while branch nested within tree identity ($n = 20$) was included as a random effect. Three separate models were created for the following response variables: count of abaxial leaf veins with mites, herbivory, and necrosis. Code for all analyses and raw data files are available on the Dryad Digital Repository (<https://doi.org/10.5061/dryad.4f4qrfjb4>).

RESULTS

Trees exhibited significant variation in the average number of abaxial leaf vein axils with hair tufts: average 2.4 ± 1.3 SD hair tufts across 20 trees (Fig. 3; generalized linear mixed model, F -value = 10.29, $df = 19$, $P < 0.001$). Overall, there was a significant association between mites and abaxial hair tufts—the odds of mites being present were 9.4 times greater

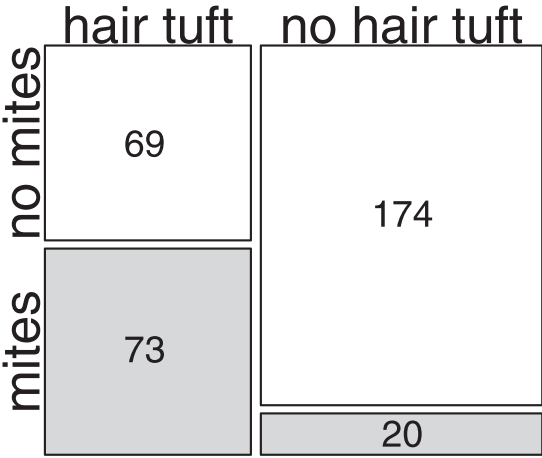


FIG. 4. The distribution of mites in leaf vein axils with and without hair tufts. Numbers indicate the total leaf count within each category.

when abaxial hair tufts were present (Fig. 4; mixed effects logistic regression, estimated log odds ratio 9.4 with 95% CI of 5.2–16.7, $P < 0.001$).

After experimentally removing hair tufts, the number of leaf vein axils containing mites was reduced by 31% relative to the control treatment (Fig. 5; generalized linear mixed model, $\chi^2 = 6.96$, $df = 1$, $P = 0.008$). There was no significant difference in the number of leaf axils with herbivory or necrosis in the control versus removal treatment (Fig. 5, generalized linear mixed model, $\chi^2 = 0.04$, 0.03 , $df = 1,1$, $P = 0.828$, 0.865 for herbivory and necrosis respectively).

DISCUSSION

These results support the hypothesis that leaf hair tufts on *Quercus agrifolia* function as domatia for mites: mites were more likely to occupy leaf vein axils with than without hair tufts, and the removal of hair tufts led to a reduction in mite abundance. We conclude that leaf hair tufts on *Q. agrifolia* likely

benefit mites, however, the specific mechanism remains untested. In other plant species, domatia were found to benefit mites by reducing desiccation (e.g., *Viburnum tinus*, Grostal and O'Dowd 1994) or by providing protection from predators (e.g., *Vitis riparia* (Michx.) A.Gray, Norton et al. 2001; *Capsicum annuum* L., Faraji et al. 2002). Whether similar mechanisms are occurring in *Q. agrifolia* will require further study.

Our results do not support the hypothesis that *Q. agrifolia* benefits from the presence of mites: when hair tufts were experimentally removed there was no impact on leaf herbivory or necrosis. This contrasts with other plant species where predatory mites were found to reduce damage from pests (e.g., *Gossypium hirsutum* L., Argawal and Karban 1997; *Cupania vernalis* Cambess., Romero and Benson 2004) and fungivorous mites were found to reduce powdery mildew (e.g., *Vitis riparia* A.Gray, Norton et al. 2000; Weber et al. 2016). In the present study, two commonly observed mites were identified to family: Phytoseiidae and Tydeidae. Family Phytoseiidae contains predatory mites that are efficient predators of spider mites, gall mites, and other plant pests (David Walter, University of the Sunshine Coast, Queensland, Australia, personal communication). Family Tydeidae contains both fungivorous and predatory mites.

While this system may indeed represent a commensalism where mites benefit from *Q. agrifolia*, but *Q. agrifolia* does not benefit from mites, we cannot yet rule out a mutualistic relationship. First, our experimental treatments only reduced mite daytime abundance by 36%. Because mites are active at night and are known to move between branches (Weber, personal observation), it could be that our experimental branches (containing ca. 5-15 leaves) were not large enough to restrict movement of mites during the night from adjacent untreated branches. Secondly, the benefits provided by mites may only occur during a specific time of year or under specific environmental conditions not captured during our ca. 10-week study interval in spring of 2019. For

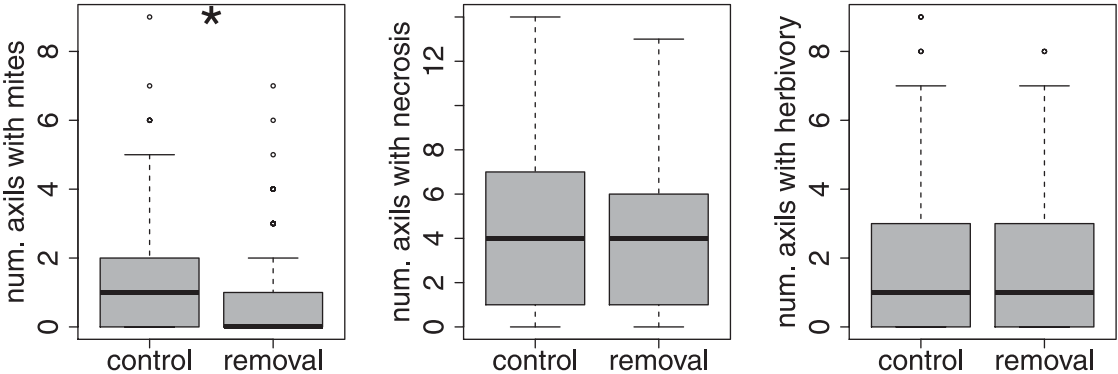


FIG. 5. Number of leaf vein axils with mites, necrosis (due to fungal or bacterial pathogens), and herbivory in control vs. hair tuft removal treatments. An asterisk (*) signifies $P < 0.05$.

example, in rare outbreak conditions of a pathogen, mite domatia may impact fungal damage (Norton et al. 2000), even if there is no detectable impact in non-outbreak years. We recently confirmed the presence of powdery mildew on an individual of *Q. agrifolia* about 10 miles west of our study site in the town of Los Osos, CA.

Evolutionary Implications

The evolution of domatia in the oak family (Fagaceae) remains largely unexplored. At least 25 oak species from subsections Lobatea, Quercus, Cerris and Ilex are known to have hair tufts, five species from subsections Quercus and Cyclobalanopsis lack hair tufts, while two species are putatively polymorphic: *Q. robur* and *Q. serrata* (Brouwer and Clifford 1990; O'Dowd and Pemberton 1994, 1998; O'Dowd and Willson 1997). Since no systematic review of domatia across oaks has yet been made, it is unclear whether domatia have evolved once or multiple times across this family.

Across all angiosperms, domatia likely evolved hundreds of times (O'Dowd and Willson 1989), yet few studies have directly examined natural selection and evolution of this key trait involved in mediating plant–arthropod interactions (but see Agrawal and Karban 1997). Within our study population, phenotypic variation in hair tuft number was present across trees. If this variation is heritable, it would provide the raw fuel for evolution and merit further study. While estimating selection in such a long-lived organism may seem unfeasible, oak tree seedlings and juvenile plants often exhibit hair tufts (Grossenbacher, personal observation). Thus, it may be feasible to estimate heritability and observe selection (at least during early life history stages) in *Q. agrifolia*, making it a system worth considering for further study.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for their helpful suggestions, David Walter for mite identifications, and Ryan Briscoe Runquist, Jenn Yost, Matt Ritter, and Brian Green for discussion and comments on an early manuscript draft. Weber was supported by NSF DEB-1831164. Additional support was provided by The Frost Fund at Cal Poly.

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