

Foliar nutrients explain goldspotted oak borer, *Agrilus auroguttatus*, adult feeding preference among four California oak species

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Abstract

Adults of the invasive goldspotted oak borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), consumed foliar weight in no-choice feeding tests of, in descending order, California black oak *Quercus kelloggii* Newb., Engelmann oak, *Quercus engelmannii* Greene, coast live oak, *Quercus agrifolia* Née, and canyon live oak, *Quercus chrysolepis* Liebm. (Fagaceae). Furthermore, significantly more foliar area was consumed of *Q. kelloggii* than of *Q. chrysolepis*. In dual-choice feeding tests with isolated leaf disks, *A. auroguttatus* consumed significantly more foliar weight and area of *Q. kelloggii* relative to the other three oak species, and more foliar weight of *Q. agrifolia* than of *Q. chrysolepis*. In dual-choice feeding tests with leaves on small branches, *A. auroguttatus* consumed more foliar weight of *Q. kelloggii* than of *Q. engelmannii* and *Q. agrifolia*. Thus, multiple experiments suggested that adults of *A. auroguttatus* preferred the foliage of *Q. kelloggii* over that of the other three oak species, and among the other three species they did not appear to have a strong feeding preference. Factor analysis reduced the quantities of 13 foliar nutrients into two new variables (factor 1 and factor 2). Factor 1 was weighted heavily on the quantities of nitrogen, sulfur, phosphorus, potassium, zinc, and copper, whereas factor 2 was weighted heavily on the quantities of zinc, iron, and aluminum. Factor 1 varied by oak species, with *Q. kelloggii* having a higher factor 1 nutrient content than the other three species. Factor 2 response was higher in *Q. kelloggii*, *Q. agrifolia*, and *Q. engelmannii* than in *Q. chrysolepis*. The collective effects of four macronutrients (nitrogen, sulfur, phosphorus, and potassium) and two micronutrients (zinc and copper) suggest that these might be the nutrients directing preferential feeding of *A. auroguttatus* adults on the foliage of *Q. kelloggii*. Leaf toughness might also play an important role in feeding preference. Female *A. auroguttatus* did not show an ovipositional preference among the four oak species.

Introduction

Since 2002, the invasive stem-infesting goldspotted oak borer, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), has caused oak mortality in San Diego County, CA, USA (Coleman et al., 2012), although the direct link between *A. auroguttatus* and oak mortality was not established until 2008 (Coleman & Seybold, 2008). In CA, oak

mortality has been frequently observed on red oaks (Fagaceae, section *Lobatae*: coast live oak, *Quercus agrifolia* Née, and California black oak, *Quercus kelloggii* Newb.) and on a taxonomically intermediate oak species (section *Protobalanus*: canyon live oak, *Quercus chrysolepis* Liebm.). Oak mortality caused by *A. auroguttatus* has not been reported on a white oak (section *Quercus*: Engelmann oak, *Quercus engelmannii* Greene) (Coleman & Seybold, 2008, 2011; Coleman et al., 2012), and *A. auroguttatus* rarely colonizes this tree species (Coleman et al., 2012). Similar colonization patterns in red and white oaks have been observed for *A. auroguttatus* in its native range in southeastern Arizona (Coleman & Seybold, 2011). The underlying

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mechanism for these host relationships and damage patterns remain unknown.

Agrilus auroguttatus undergoes four instars before pupating (Haavik et al., 2013). Upon emergence from pupae, *Agrilus* spp. adults such as emerald ash borer, *Agrilus planipennis* Fairmaire, generally feed on host plant foliage for 5–7 days before mating (Poland & McCullough, 2006; Flint et al., 2013). Females feed for an additional week before ovipositing on the bark surface of the stem and larger branches (Poland & McCullough, 2006; Flint et al., 2013). The larvae of these species mine in the phloem and feed extensively at the interface between the xylem and phloem. This feeding causes a decline in tree health and leads eventually to tree mortality (Coleman et al., 2011). Adult *Agrilus* spp. feeding activity on foliage does not appear to have a major impact on tree health (Flint et al., 2013). It is not known whether adult *A. auroguttatus* feed upon foliage from the same individual tree or tree species that they emerge from, or whether females oviposit on the same individual tree or tree species that they feed on as adults. Given that host plant quality usually positively affects insect fecundity (Awmack & Leather, 2002), natural selection should result in insects feeding on high-quality host plants, assuming the absence of other influences (e.g., intraspecific competition or predation risk). Moisture and nutrient content are two important determinants of this food quality (White, 1993; Awmack & Leather, 2002; Chen & Poland, 2010; Chen et al., 2011).

No studies have been conducted to determine feeding or ovipositional preferences of *A. auroguttatus* adults for host foliage from various oak species. The objectives of this study were to investigate: (1) whether there were *A. auroguttatus* adult feeding preferences for foliage among *Q. agrifolia*, *Q. chrysolepis*, *Q. engelmannii*, and *Q. kelloggii* – and if there were, whether these could be explained by oak foliage nutrients and (2) whether *A. auroguttatus* adult females oviposited differentially on the four oak species. We hypothesized that *A. auroguttatus* adults (1) prefer to feed on *Q. agrifolia*, *Q. kelloggii*, and *Q. chrysolepis* rather than on *Q. engelmannii* based on oak tree colonization and mortality patterns in the field; (2) have their feeding preference linked to higher nutritional levels in foliage of particular oak species; and (3) have their female ovipositional preference corresponding with their feeding preference.

Materials and methods

Insects

Because *Q. agrifolia* is the most frequently and heavily infested species, *A. auroguttatus* adults were collected in the prepupal stage from infested *Q. agrifolia* located in

William Heise County Park (33.03997°N, 116.58956°W, elevation 1 384 m; Julian, San Diego County) and in Kitchen Creek Canyon (32.77823°N, 116.44879°W, elevation 1 371 m) and Corral Canyon [32.72733°N, 116.54075°W, elevation 1 043 m; both Descanso Ranger District, Cleveland National Forest (CNF), San Diego County]. Although all experimental insects were collected on *Q. agrifolia*, our results (below) indicated no feeding bias for *Q. agrifolia*. Bark was removed from infested trees with hatchets and *A. auroguttatus* prepupae were harvested in bark and phloem pieces. These collections occurred from 2 April to 23 May 2012 from five trees. Samples were returned to the laboratory and beetles were allowed to complete development and emerge in screen observation cages (ca. 5.4 m³; BioQuip Products, Rancho Dominguez, CA, USA) at ambient conditions inside the laboratory. Adults were hand collected from the walls of these cages. Due to the scarcity of adults, mixed sexes were used in all tests except the ovipositional tests in which only females were used. Female adults were identified by the absence of the median groove on the first abdominal segment; this groove is present on the males (Coleman & Seybold, 2010).

Oak foliage samples

Foliage and branch samples of *Q. agrifolia*, *Q. chrysolepis*, *Q. engelmannii*, and *Q. kelloggii* were collected on 5 and 19 June and 17 July 2012 from the Descanso Ranger District and surrounding communities by using hand and pole pruners. Specifically, foliage and branch samples of *Q. agrifolia* were collected from Horsethief Canyon (CNF), Mt. Laguna (CNF), and Bell Bluff (Japatal Valley, San Diego County). *Quercus engelmannii* samples were collected from Roberts Ranch (CNF) and Bell Bluff. *Quercus chrysolepis* and *Q. kelloggii* samples were collected from Mt. Laguna (CNF). At each collection site, six trees of each species without any evidence of *A. auroguttatus* damage (i.e., no crown dieback, emergence holes, bark staining, or woodpecker damage; Coleman et al., 2011; Hishinuma et al., 2011) were sampled. If the same area was visited at subsequent sampling points, foliage and branches were collected from previously unsampled trees. These samples were transported to the laboratory in ziploc bags in an ice-filled cooler. Leaves from the foliage samples were used immediately for bioassay or stored in a refrigerator at 4 °C and bioassayed within a week. Mature leaves were used in all bioassays.

Specific leaf weights

Specific leaf weight (SLW) or leaf mass per area is used widely as an indicator of leaf toughness (Steinbauer, 2000; Cornelissen et al., 2003). Specific leaf weights of the four

oak species were calculated from six data pairs of foliar weight (Y) and foliar area (X) collected by measuring the fresh weight and foliage area of entire leaf samples from each species. To quantify foliar weight from foliar area, a regression line ($Y = b + aX$) was also constructed from six replicated measurements for each species. The area of foliage was quantified with a digital camera and digital imaging software – ‘Image Processing and Analysis in Java’ (ImageJ), version 1.34s; available in the public domain at <http://rsbweb.nih.gov/ij/>).

No-choice feeding tests (leaf disks)

Leaf disks (1.64 cm diameter, 2.13 cm²) were cut by hand with a bit from a Deluxe Plug Cutting Kit (Woodcraft, Parkersburg, WV, USA) from foliage from each of the four oak species (four replicates of each test). A leaf disk from one species was placed in the center of a 6-cm-diameter petri dish, and a 1- to 2-day-old adult beetle was placed directly on the disk and allowed to feed for 48 h. At the end of the experiment, the area of foliage and foliar weight consumed were quantified by imaging and regression analysis as described above.

Dual-choice feeding tests (leaf disks)

Dual-choice treatments were as follows: (1) *Q. agrifolia* vs. *Q. kelloggii*; (2) *Q. agrifolia* vs. *Q. chrysolepis*; (3) *Q. agrifolia* vs. *Q. engelmannii*; (4) *Q. kelloggii* vs. *Q. chrysolepis*; (5) *Q. kelloggii* vs. *Q. engelmannii*; and (6) *Q. chrysolepis* vs. *Q. engelmannii* (eight replicates of each test). Leaf disks were cut from each oak species with the same dimensions and drill bit described above. Leaf disks from the two oak species in each test were placed ca. 1 cm from the rim of a 6-cm-diameter petri dish and ca. 2.5 cm apart along the diameter of the dish. Disks from the two oak species were placed at random into these positions for each trial and a 1- to 2-day-old male or female *A. auroguttatus* was placed exactly between the two disks on the floor of the dish and allowed to feed for 48 h. Foliar area and foliar weight consumed were quantified as described above. Percentage of foliar area consumed was calculated as foliar area consumed divided by 2.13, the total area of a leaf disk ($\times 100\%$).

Dual-choice feeding tests (branches)

Tests with small branches (six replicates of each test) were conducted in plastic box cages (27 × 9.5 × 19.4 cm; Pioneer Plastics, Dixon, KY, USA). One small branch (ca. 15–19 cm long) from each oak species was first inserted through a small hole (ca. 0.5 cm diameter) into a 15-ml glass vial, which was filled with water to maintain the turgidity of leaves. Each test branch from *Q. agrifolia*, *Q. chrysolepis*, and *Q. engelmannii* contained ca. 10–20

leaves, whereas each branch from *Q. kelloggii* contained ca. 5–8 leaves. Test branches from two oak species were then placed randomly at each end of a cage (ca. 2 cm from the end and 3 cm from the sides). A 1- to 2-day-old male or female adult *A. auroguttatus* was placed on the floor in the center of the cage and allowed to feed for 5 days. Foliar area (as a percentage) and foliar weight consumed were quantified as described above. Due to limited availability of adult *A. auroguttatus*, the choice *Q. kelloggii* vs. *Q. chrysolepis* was not offered.

Moisture and nutrient determination

To determine moisture and nutrient contents of the foliage (six replicates of each measurement for each species), leaves (in amounts ranging from 23 to 70 g) were collected from all foliage samples and oven dried at 50 °C for 72 h. Leaves were weighed before (fresh weight) and after (dry weight) drying, and moisture content was calculated as the difference between the fresh and dry weight divided by the fresh weight. Foliage samples were then shipped to A&L Great Lakes Laboratories (Fort Wayne, IN, USA) for nutrient analysis. The leaves were digested for analysis by using an Open Vessel Microwave (procedure reference no. SW846-3051A), and the nutrients were quantified by using the Inductively Coupled Argon Plasma technique (ICAP; procedure reference no. AOAC 985.01). Nutrients analyzed included seven macronutrients (nitrogen, sulfur, phosphorus, potassium, magnesium, calcium, and sodium) and six micronutrients (boron, zinc, manganese, iron, copper, and aluminum). The units of the results reported by the vendor were % dry weight or p.p.m., depending on the nutrient.

Dual-choice oviposition tests

Oak branch sections (ca. 12 cm long, 2–3 cm in diameter) were cut from the four oak species. Both ends of a branch section were wrapped with Parafilm (Sigma-Aldrich, St. Louis, MO, USA) to prevent moisture loss. In the field, *A. auroguttatus* eggs are laid in the bark crevices of the main stem and branches (Flint et al., 2013). To simulate bark crevices and to facilitate oviposition by female *A. auroguttatus* on the smooth bark surface of these small branch sections, the bark surface of each branch section was wrapped spirally with a 0.5-cm-wide nylon ribbon (Berwick Industries, Berwick, PA, USA) (Duan et al., 2012). Branch sections from two oak species were then placed vertically at the two ends of the plastic cages described above (initially 12 replicates of each test). A twig with leaves of a third oak species (chosen at random from the remaining two species) was inserted into a glass vial before being placed in the middle of the cage as a food supply for the female *A. auroguttatus* in the test. A ca. 10-day-

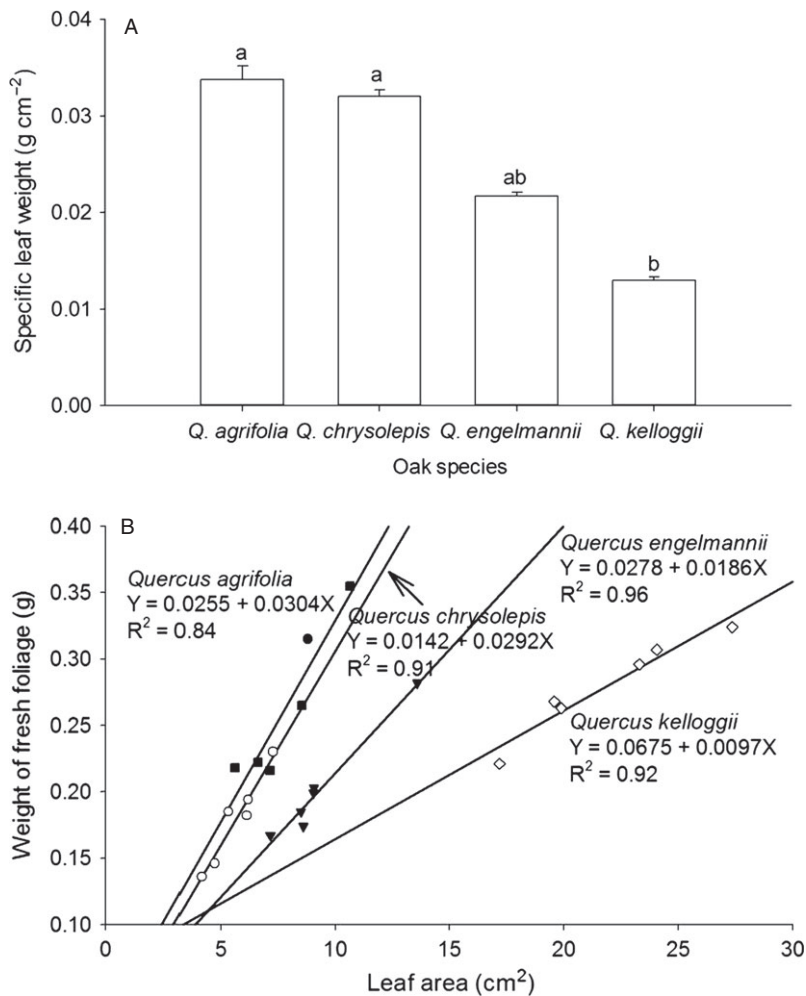


Figure 1 (A) Mean (+ SE) specific leaf weights and (B) regression of foliage weight (Y) vs. foliar area (X) for four southern California oak (*Quercus*) species (n = 6 for both analyses). Different letters above the histogram bars in panel A indicate significant differences among means (test as described in Elliott & Hynan, 2011).

old female was released on the floor at the center of the cage and allowed to oviposit for a week. Eggs that had been laid on branch sections and cage walls were counted at the end of the experiment. The cage walls were divided into half and each half was assigned to the most proximal of the experimental branch sections. Choice tests included *Q. agrifolia* vs. *Q. kelloggii*, *Q. agrifolia* vs. *Q. engelmannii*, *Q. chrysolepis* vs. *Q. kelloggii*, and *Q. chrysolepis* vs. *Q. engelmannii*.

Statistical analyses

Specific leaf weights could not be normalized and were analyzed by a non-parametric Kruskal-Wallis test with multiple mean comparisons following the method described by Elliott & Hynan (2011). No-choice feeding test data were checked for normality with Kolmogorov-Smirnov's D statistic and for variance homogeneity with Levene's test before being subjected separately to ANOVA. Data for foliar area consumed were not normally distrib-

uted and were transformed by using the Box-Cox method ($\lambda = -0.10$) (Proc TRANSREG; SAS Institute, 2009). Means were separated with Tukey's honestly significant difference (HSD) test, if the null hypothesis of no significant difference among species was rejected. In the dual-choice feeding tests, the differences between species in foliar area and weight consumed were analyzed with paired t-tests if the differences were normally distributed. Otherwise, they were analyzed by using the Wilcoxon signed rank test (Proc UNIVARIATE; SAS Institute, 2009).

Normality and variance homogeneity of moisture and nutrient content data were checked as described above. Sulfur, phosphorus, potassium, sodium, zinc, iron, copper, and aluminum data sets were not normally distributed and were transformed by using the Box-Cox method, with λ 's of -1.75 , -2.50 , -1.25 , -3.00 , -0.25 , -0.75 , 0.50 , and -0.50 , respectively. Data for moisture content and each nutrient were then analyzed separately with one-way ANOVA. Means were separated with

Tukey's HSD if the null hypothesis of no significant difference among species was rejected. Factor analysis can reduce the original number of variables and create fewer, mutually independent new variables (i.e., factors) (SAS Institute, 2009). This technique was applied to the nutrient data following the methods of Chen (2013). Both principal component analysis and maximum likelihood analysis factor-extracting methods were used to determine the optimal number of factors to retain. The rotation method was the orthogonal varimax rotation (SAS Institute, 2009). The two factors retained were normally distributed with homogeneous variances. These were further analyzed with one-way ANOVA. Means were separated with Tukey's HSD if the null hypothesis of no significant difference among species was rejected.

In the dual-choice oviposition tests, the differences between oak species in the number of eggs laid on branch sections and on the nearest cage walls assigned to each branch section, and in total number of eggs were analyzed separately with paired t-tests if the differences were normally distributed. Otherwise, they were analyzed by using the Wilcoxon signed rank test (Proc UNIVARIATE; SAS Institute, 2009). All statistical analyses were conducted with a critical threshold of $\alpha = 0.05$.

Results

Specific leaf weights of four oak species

Oak species significantly affected SLWs ($\chi^2 = 19.85$, d.f. = 3, $P < 0.001$). Specific leaf weights of *Q. agrifolia* and *Q. chrysolepis* did not differ from that of *Q. engelmannii*, but they were greater than that of *Q. kelloggii* (Figure 1A). Although SLW of *Q. kelloggii* did not differ from that of *Q. engelmannii*, it was approximately half that of *Q. engelmannii* (Figure 1A). The regression line equations [foliar weight = $b + a$ (foliar area)] and the adjusted R^2 values for all four oak species are given in Figure 1B (in all cases, the regression coefficient a was significantly different from zero and b was not).

No-choice feeding tests (leaf disks)

Foliar area consumed by *A. auroguttatus* varied significantly with oak species ($F_{3,12} = 4.94$, $P < 0.05$) as did foliar weight consumed ($F_{3,12} = 67.65$, $P < 0.01$). Both in terms of area (Figure 2A) and in terms of weight (Figure 2B) the beetles consumed most leaf material of *Q. kelloggii* and the least of *Q. chrysolepis*.

Dual-choice feeding tests (leaf disks)

Relatively more *Q. kelloggii* foliar area was consumed than of any other species (*Q. kelloggii* vs. *Q. agrifolia*: $t = 2.59$; vs. *Q. chrysolepis*: $t = 3.01$; vs. *Q. engelmannii*: $t = 3.14$,

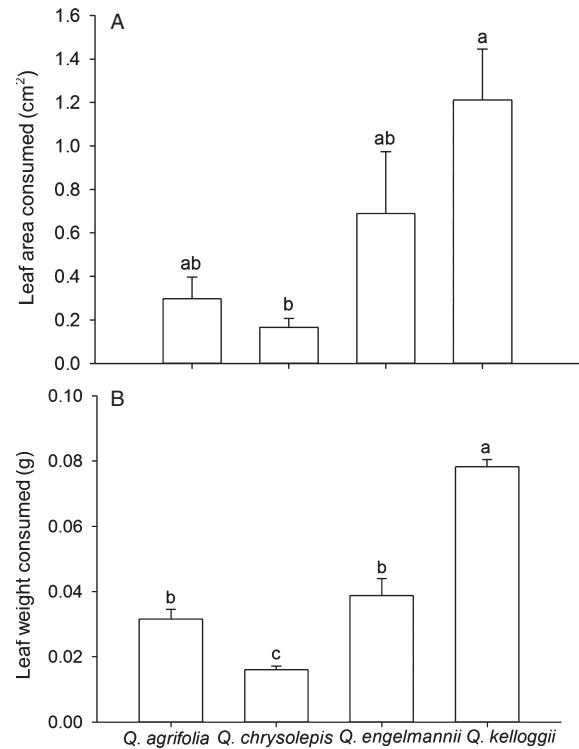


Figure 2 No-choice test of *Agrilus auroguttatus* adults feeding on small leaf disks of one of four southern California oak (*Quercus*) species ($n = 4$). Mean (+ SE) area (A) and weight (B) of foliage consumed. Different letters above the bars indicate significant differences among means (Tukey's HSD; $P < 0.05$).

all $P < 0.05$) (Figure 3A). Also, more *Q. kelloggii* foliar weight was consumed than in any other species (*Q. kelloggii* vs. *Q. agrifolia*: $t = 6.15$; vs. *Q. chrysolepis*: $t = 4.15$; vs. *Q. engelmannii*: $S = 10.5$, all $P < 0.05$) (Figure 3B). *Agrilus auroguttatus* fed more on *Q. agrifolia* foliage than on *Q. chrysolepis* foliage in the dual-choice test ($t = 2.67$, $P < 0.05$) (Figure 3B).

Dual-choice feeding tests (branches)

There were no significant differences in the relative amounts of foliar area consumed between oak species in species pairs in any of the five tests with small branches (Figure 4A). However, more foliar weight of *Q. kelloggii* was consumed vs. *Q. agrifolia* ($t = 10.64$, $P < 0.01$) or vs. *Q. engelmannii* ($t = 5.09$, $P < 0.01$) (Figure 4B).

Moisture and nutrient determination

Leaf moisture content varied significantly across oak species ($F_{3,20} = 10.89$, $P < 0.01$). The moisture contents of *Q. kelloggii*, *Q. chrysolepis*, and *Q. engelmannii* were not different, but they were all greater than that of *Q. agrifolia* (Table 1). Quantities of several nutrients also varied

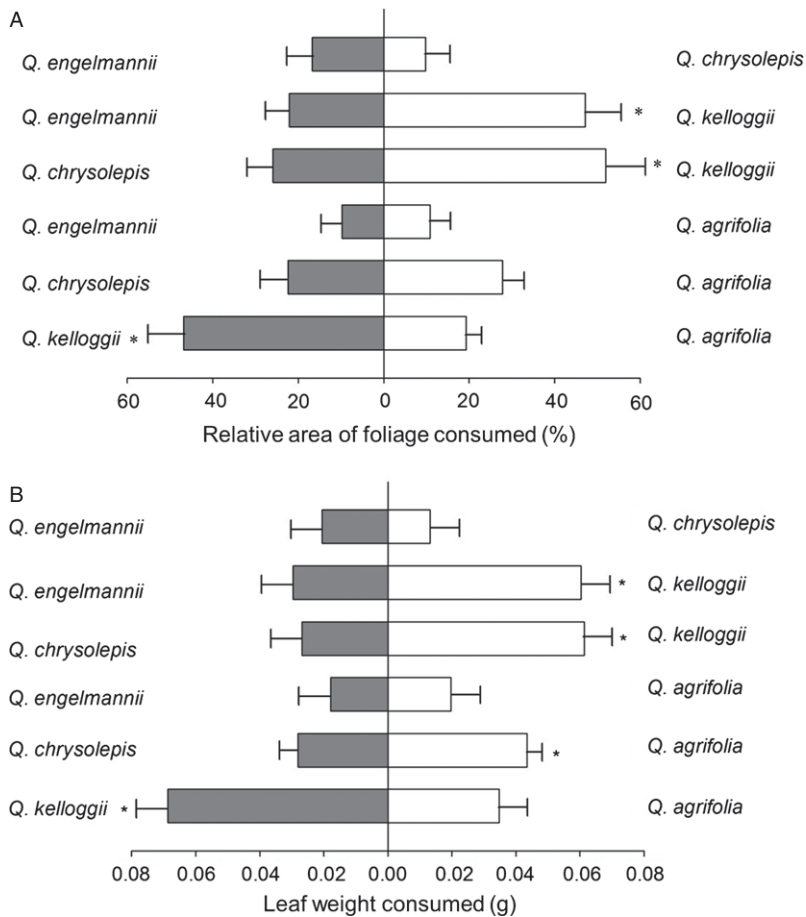


Figure 3 Dual-choice test of *Agrilus auroguttatus* adults feeding on small leaf disks from four southern California oak (*Quercus*) species (n = 8). Mean (+ SE) (A) relative area and (B) weight of foliage consumed. Asterisks indicate significant preference (t-test or Wilcoxon signed rank test: *P<0.05).

significantly across oak species, including nitrogen ($F_{3,20} = 53.90$, $P < 0.01$), magnesium ($F_{3,20} = 7.71$, $P < 0.01$), calcium ($F_{3,20} = 7.57$, $P < 0.01$), boron ($F_{3,20} = 3.58$, $P < 0.05$), and manganese ($F_{3,20} = 12.03$, $P < 0.01$) (Table 1). Factor analysis reduced the 13 nutritional variables into two new variables (i.e., factors 1 and 2) (Table 2). The first weighted heavily on nitrogen, sulfur, phosphorus, potassium, zinc, and copper (weights exceeded 0.5), whereas the second weighted heavily on sulfur, zinc, iron, and aluminum (weights exceeded 0.5). Factor 1 varied significantly across oak species, with content of factor 1 nutrients higher in *Q. kelloggii* than in the other three species (Table 2). Factor 2 also varied significantly across oak species, with content of factor 2 nutrients higher in *Q. agrifolia* than in *Q. chrysolepis* (Table 2). Content of factor 2 in *Q. kelloggii* did not differ from that in the other three species.

Dual-choice oviposition tests

In general, more eggs were generally deposited on the walls of both ends of the cages than on the branch sections (Figure 5). Only in the case of *Q. engelmannii* in the compara-

tive studies with *Q. agrifolia* or *Q. chrysolepis* was oviposition higher on the branch sections than on the corresponding cage wall (Figure 5B and D). There were no significant differences in the numbers of eggs laid on branches of different oak species, regardless of whether oviposition occurred under the ribbon on the branch bark surface, on the associated cage wall, or on both combined (Figure 5).

Discussion

Moisture and nutritional content are two important factors that drive feeding behavior of insects (Slansky & Scriber, 1985; White, 1993; Chen et al., 2011). Nutritional elements such as nitrogen directly affect insect fecundity (Dixon, 1970; Awmack & Leather, 2002). Nutritional quality of host plants might also determine insect reproductive strategies such as the timing of reproduction (Dixon, 1971) and the size of eggs (Leather & Burnand, 1987). Therefore, insect females might face selection to choose high-quality food plants that have higher levels of moisture and/or key nutrients.

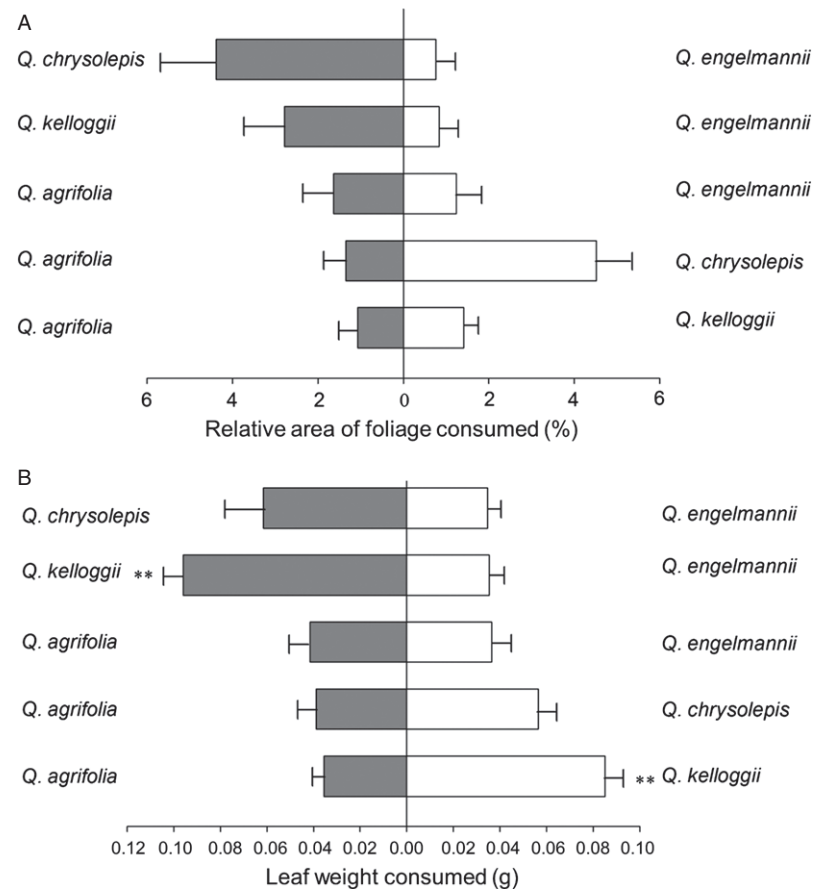


Figure 4 Dual-choice test of *Agrilus auroguttatus* adults feeding on leaves on small branches from four southern California oak (*Quercus*) species (n = 6). Mean (+ SE) (A) relative area and (B) weight of foliage consumed. Asterisks indicate significant preference (t-test or Wilcoxon signed rank test: **P<0.01).

Table 1 Mean (\pm SE; n = 6) moisture and nutrient content of the foliage of four oak (*Quercus*) species in San Diego County, CA, USA

Item	<i>Q. agrifolia</i>	<i>Q. kelloggii</i>	<i>Q. chrysolepis</i>	<i>Q. engelmannii</i>
Moisture (%)	21.85 \pm 1.53b	32.37 \pm 0.81a	34.01 \pm 1.31a	28.74 \pm 2.44a
Nitrogen (%)	1.51 \pm 0.03b	1.98 \pm 0.04a	1.17 \pm 0.05c	1.32 \pm 0.07b
Phosphorus (%)	0.10 \pm 0.00	0.20 \pm 0.02	0.11 \pm 0.01	0.10 \pm 0.00
Potassium (%)	0.54 \pm 0.02	1.14 \pm 0.09	0.61 \pm 0.01	0.80 \pm 0.07
Sulfur (%)	0.11 \pm 0.00	0.15 \pm 0.01	0.09 \pm 0.00	0.10 \pm 0.01
Magnesium (%)	0.29 \pm 0.02a	0.22 \pm 0.02b	0.22 \pm 0.02b	0.18 \pm 0.01b
Calcium (%)	0.97 \pm 0.09b	1.18 \pm 0.07ab	1.52 \pm 0.09a	1.06 \pm 0.09b
Sodium (%)	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00	0.01 \pm 0.00
Boron (p.p.m.)	49.50 \pm 6.85ab	56.50 \pm 2.79ab	64.17 \pm 11.67a	33.00 \pm 2.52b
Zinc (p.p.m.)	36.83 \pm 5.10	34.33 \pm 3.21	18.83 \pm 1.35	18.83 \pm 1.28
Manganese (p.p.m.)	822.33 \pm 111.19a	286.83 \pm 97.31bc	554.00 \pm 58.08ab	201.67 \pm 32.10c
Iron (p.p.m.)	279.50 \pm 71.13	141.00 \pm 25.65	87.00 \pm 6.09	114.5 \pm 10.68
Copper (p.p.m.)	5.00 \pm 0.44	6.67 \pm 0.21	5.00 \pm 0.26	4.83 \pm 0.31
Aluminum (p.p.m.)	194.50 \pm 49.11	100.33 \pm 16.80	66.17 \pm 7.99	90.50 \pm 8.01

Means within a row followed by different letters are significantly different (Tukey's HSD: P<0.05). Means were calculated from untransformed data.

Agrilus auroguttatus adults consumed more foliar area and weight of *Q. kelloggii* than of *Q. agrifolia*, *Q. chrysolepis*, or *Q. engelmannii* in this study. Furthermore, adult longevity was enhanced when *A. auroguttatus* fed on

foliage of *Q. kelloggii* in comparison to eight other species of oaks and an alder (*Alnus rhombifolia* Nuttall, Betulaceae) (LJ Haavik, unpubl.). *Agrilus auroguttatus* adults generally did not differentiate among foliage

Table 2 Coefficients (i.e., weights) of the original nutrient variables on the two mutually independent new variables after factor analysis of nutritional content of the foliage of four oak (*Quercus*) species in San Diego County, CA, USA

Elemental nutrient	Factor 1 (58.11%)	Factor 2 (30.44%)
Nitrogen	0.8664	0.2864
Sulfur	0.7905	0.5019
Phosphorus	0.9778	-0.1034
Potassium	0.6332	-0.0558
Magnesium	-0.0049	0.2578
Calcium	-0.0820	-0.2466
Sodium	-0.0785	0.1100
Boron	0.0941	0.0716
Zinc	0.5593	0.5180
Manganese	-0.0938	0.2474
Iron	0.1569	0.8823
Copper	0.7911	0.1024
Aluminum	0.0894	0.9405
ANOVA		
F	27.88	
		4.03
d.f.	3,20	3,20
P	<0.01	<0.05
Multiple comparison ¹		
<i>Q. agrifolia</i>	a	A
<i>Q. chrysolepis</i>	a	B
<i>Q. engelmannii</i>	a	AB
<i>Q. kelloggii</i>	b	AB

Maximum likelihood factor-extracting method and orthogonal varimax rotation were used in the factor analysis.

¹Significant difference among species is indicated by different lower case (factor 1) and uppercase (factor 2) letters (Tukey's HSD: $P < 0.05$).

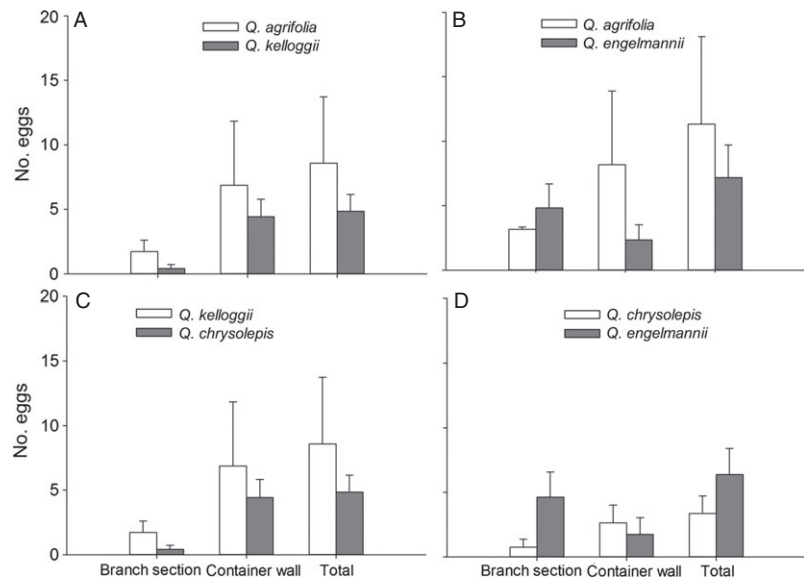
samples of the three less-preferred oak species that we tested. These feeding preferences were probably not due to foliar moisture content alone because our measurements showed that the moisture content of *Q. kelloggii* did not differ from that of *Q. chrysolepis* or *Q. engelmannii* (Table 1). However, the preferences may be related to higher quantities of four macronutrients (i.e., nitrogen, sulfur, phosphorus, and potassium) and two micronutrients (i.e., zinc and copper) that are found in *Q. kelloggii*. The positive association of multiple nutrients with herbivore populations has been demonstrated (Joern et al., 2012). Moisture content might still be an important factor in *A. auroguttatus* feeding preference in instances where all nutrients are at the same levels or in the context of other factors related to leaf anatomy or specialized metabolism.

Our SLW tests showed that foliage of *Q. kelloggii* was less tough than that of *Q. agrifolia*, *Q. chrysolepis*, or *Q. engelmannii*. This might also have contributed to adult

A. auroguttatus feeding preferentially on *Q. kelloggii* foliage. Leaf toughness affects feeding preference of many herbivores, in particular those non-adapted ones (Nichols-Orians & Schultz, 1989, 1990; Casher, 1996). For instance, the first instar California oakworm, *Phryganidia californica* Packard, prefers the softer adaxial surface over the tougher abaxial surface when it initiates feeding (Casher, 1996). *Agrilus planipennis*, a closely related species to *A. auroguttatus*, also tends to feed more on the softer foliage of *Fraxinus pennsylvanica* Marsh. and *Fraxinus americana* L. than on the tougher foliage of *Fraxinus nigra* Marsh. (Chen & Poland, 2010).

In southern California, oak trees are killed by multiple years of consistent *A. auroguttatus* larval attack (Coleman et al., 2011; Flint et al., 2013). The selection of the larval feeding host is carried out in the field through the ovipositional behavior of the female. In our laboratory study, the ovipositing females of *A. auroguttatus* did not differentiate among branches of the four oak species that we tested (Figure 5). *Agrilus auroguttatus* has been observed to colonize and develop in *Q. agrifolia*, *Q. kelloggii*, and *Q. chrysolepis* at far higher rates than in *Q. engelmannii* in oak woodlands (Coleman & Seybold, 2008, 2011; Coleman et al., 2012). With other conditions being identical and assuming a positive association between foliar preference/nutrient status and detection of phloem nutrient status through bark surface chemistry, *A. auroguttatus* females were expected to prefer *Q. kelloggii* for oviposition. The disparities between laboratory and field observations of adult *A. auroguttatus* oviposition preference might be explained in part by the laboratory conditions under which *A. auroguttatus* females were forced to oviposit. *Agrilus auroguttatus* eggs are laid primarily in bark crevices on tree stems and the larger branches (Flint et al., 2013; Haavik et al., 2013); branch sections used in the laboratory study were much smaller in diameter and were covered with ribbon to simulate bark crevices. In the field, larger oak trees (>13 cm in diameter at breast height) experience higher rates of *A. auroguttatus* mortality than smaller trees (Coleman et al., 2012). The higher number of eggs laid on cage walls in our experiment might also be an indicator of the unnatural habitat presented for assay in the laboratory. Alternatively, other ecological conditions or selective forces on *A. auroguttatus* oviposition might account for the disparities. Selection of a low- instead of a high-quality host plant is a frequent behavior carried out to minimize the risk of predation/parasitism (Damman, 1987; Hacker & Bertness, 1995; Ballabeni et al., 2001). This was best expressed as the 'enemy free space hypothesis' proposed by Thompson (1988). Both predators and parasitoids of *A. auroguttatus* have been reported in California (Coleman & Seybold, 2011; Haavik

Figure 5 Mean (+ SE) number of eggs laid by *Agrilus auroguttatus* females on branch sections of four southern California oak (*Quercus*) species and on the container wall in dual-choice tests ($n = 8$). (A) *Q. agrifolia* vs. *Q. kelloggii*; (B) *Q. agrifolia* vs. *Q. engelmannii*; (C) *Q. kelloggii* vs. *Q. chrysolepis*; and (D) *Q. chrysolepis* vs. *Q. engelmannii*. There were no significant differences among the means within a species comparison.



et al., 2012), although neither group of natural enemies appears to contribute significantly toward mortality of *A. auroguttatus* (Flint et al., 2013).

Lack of a positive correlation between female oviposition preference and offspring performance has long been recognized (Courtney & Kibota, 1990; Berdegué et al., 1998; Gripenberg et al., 2010). To investigate the correspondence of *A. auroguttatus* oviposition preference and larval performance, phloem/bark nutritional and defensive status will need to be analyzed in the context of *A. auroguttatus* larval survival and development in the four *Quercus* species. Oviposition tests with larger branch or stem sections in the laboratory or on trees in the field might also be investigated. Future research on foliage preference by adult *A. auroguttatus* might also involve an analysis of choice/no-choice assays, nutrient content, moisture content, and leaf toughness in the context of foliage harvested from trees in various crown injury classes attributed to *A. auroguttatus* (Hishinuma et al., 2011).

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