

Short communication

Textured Hive Interiors Increase Honey Bee (Hymenoptera: Apidae) Propolis–Hoarding Behavior

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Abstract

Numerous papers have shown that propolis contributes favorably to worker honey bee (*Apis mellifera* L.) immune response and colony social immunity. Moreover, resin-foraging specialists are more sensitive than pollen foragers to tactile information in the nest interior, and they respond to these stimuli by collecting more resin. In this study, we show that in-hive propolis deposition is increased, compared with nonmodified controls, with any one of the three methods for increasing textural complexity of hive wall interior surfaces: 1) plastic propolis trap material stapled to wall interior, 2) parallel saw kerfs cut into wall interior, or 3) roughening wall interior with a mechanized wire brush. Pairwise comparisons showed that propolis deposition was not significantly different among the three textural treatments; however, textural treatments interacted with time to show a more consistent benefit from plastic propolis trap material or roughened interior surface over saw kerfs. Although direct health benefits were not measured, this work shows that it is comparatively simple to increase propolis deposition above background levels by increasing textural stimuli in hive interiors.

Key words: *Apis mellifera*, honey bee health, foraging, plant resins

Foraging honey bees collect a variety of plant resins which are used at the nest to form a pliable heterogeneous amalgam known by the beekeeping term ‘propolis’. Bees apply propolis to interior surfaces of the nest, chiefly at points of comb attachment, presumably aiding in structural strength, and all over the interior walls of the cavity, forming what has been called a ‘propolis envelope’ (Seeley and Morse 1976). The propolis envelope resists wood decay fungi and water that would otherwise compromise the integrity of the cavity and fills in cracks and crevices that could harbor small nest invaders. Bees use propolis to imprison the nest-invading beetle *Aethina tumida* (Neumann et al. 2001) and to mummify the bodies of vertebrate nest invaders too large for bees to remove (Simone-Finstrom and Spivak 2010). The antibacterial properties of propolis have been known for decades (Lavie 1960).

Propolis has garnered much interest in the honey bee literature, due largely to its properties as a human health supplement (Mizrahi and Lensky 1996, Burdock 1998) and ironically to its reputation as a nuisance for gumming together hive parts used in beekeeping (Delaplane 2007). However, new knowledge on the contributions of propolis to worker bee immune response and colony social immunity makes clear that propolis deposition in the nest is adaptive and contributes positively to honey bee health at many levels of social organization (Simone et al. 2009, Evans and

Spivak 2010, Simone-Finstrom and Spivak 2010, Simone-Finstrom et al. 2017).

When investigators painted interior walls of experimental hives with an ‘artificial propolis envelope’ of propolis extract, treated colonies had overall lower bacterial loads, and workers in those colonies expressed lower levels of immune gene expression (Simone et al. 2009). Although activation of immune pathways is an adaptation for mitigating individual infections, if this activation is sustained there is an energetic cost which expresses at the colony level as a reduction in brood (Evans and Pettis 2005). A follow-up study allowed bees to make their own propolis envelopes from resins within their natural foraging range and supported the earlier evidence for reduced energetic investment in individual immune response, but failed to repeat the result for overall reduction in colony bacterial loads (Borba et al. 2015). These studies, along with unpublished work cited in Simone-Finstrom et al. (2017), suggest that reduced immune activation in propolis-treated bees is not from immune suppression by propolis, but rather reduced pathogen load in propolis-treated colonies. A leading hypothesis emerging from this work is that the collection of plant resins evolved as a colony-level adaptation for relieving workers of the need for sustaining an energetically costly immune response, especially when the colony is not being challenged by pathogens.

In vivo field studies have supported a hypothesis that the propolis envelope helps reduce colony-level pathogen burden. Compared with controls, investigators found significantly fewer dead larvae symptomatic of chalkbrood disease, causative fungus *Ascosphaera apis*, in chalkbrood-challenged colonies whose hive interiors had been painted with a propolis extract (Simone-Finstrom and Spivak 2012). A similar study was done focusing on American foulbrood, a larval disease caused by the bacterium *Paenibacillus larvae*. Compared with nontreated controls, colonies with propolis envelopes had fewer symptoms of American foulbrood, and larval food produced by nurse bees from propolis-treated colonies had higher antimicrobial activity (Borba and Spivak 2017).

Work of this kind leads naturally to interest in methods for inducing the benefits of propolis in managed colonies. There is a reason to think that resin collecting is at least partially under genetic control (Simone-Finstrom et al. 2017); resin-foraging specialists are more sensitive than pollen foragers to tactile information, specifically a gap between two plates or a rough sandpaper surface, and they respond to these stimuli by collecting more resin (Simone-Finstrom et al. 2010). Increasing tactile complexity in the hive interior therefore stimulates resin foraging, a principle already exploited in the work reported here (Borba et al. 2015, Borba and Spivak 2017), in which investigators stapled sheets of plastic commercially available propolis traps (Mann Lake, Hackensack, MN) onto interiors of hive walls.

We were interested in testing whether increased tactile stimuli can be engineered into the hive-manufacturing process, thereby providing a more direct and economical way to stimulate propolis deposition in the hive.

Methods

Twenty nucleus colonies, each stocked with five Langstroth deep brood frames, ca. 1.5-kg worker bees, and a queen were randomly distributed among five apiary sites (four colonies per site) around the metro area of Atlanta, GA. Each apiary was managed by an independent beekeeper cooperator and no closer than 19 km from another. No genetic selection criteria were applied to the queens.

At each apiary, each experimental colony was randomly assigned one of the four texturizing treatments: 1) the plastic propolis trap method used by Borba et al. (2015) and Borba and Spivak (2017) (Fig. 1), 2) five parallel horizontal saw kerfs, 7 cm apart, cut 0.3 cm deep into the surface (Fig. 2), 3) roughening the interior surface with a mechanized wire brush (Fig. 3), or 4) unmodified planed (smooth) interior surface (nontexturized control). All woodenware was sawn from pine (*Pinus* spp.) lumber.

Colonies were set up by 14 August 2016 and left free to forage on locally available resin sources. The foraging range is in the temperate Appalachian Piedmont of the Southeast United States where prominent trees include a variety of conifers (*Pinus* spp.), oaks (*Quercus* spp.), pecan (*Carya illinoensis*), red maple (*Acer rubrum*), yellow poplar (*Liriodendron tulipifera*), and diverse urban woody ornamental species. One apiary perished over the winter of 2016–2017 and its hives were reconstituted into the experiment and distributed to two other apiaries by March 2017.

During each of the weeks of 17 October 2016, 20 March 2017, 27 June 2017, and 16 September 2017, each surviving colony was inspected to measure propolis deposition. For each measurement, data were cumulative so that, for example, data for the week of 16 September 2017 represent 13 mo' worth of deposition. This time series was accounted for in our statistical analyses. Although propolis is ethanol-soluble, the size of hive parts made it impractical to



Fig. 1. Plastic propolis trap (Mann Lake, Hackensack, MN) applied to interior hive walls after (Borba et al. 2015, Borba and Spivak 2017).

dissolve, recover, and weigh propolis in a quantitative manner. This, along with the patchiness and depth irregularities of natural propolis deposition, convinced us to use a subjective scoring system to differentiate deposition among the four treatments. All bees and combs were removed from each brood box, and each of four interior surfaces of each box digitally photographed and labeled. All images were put in an online file repository to which five volunteers were granted access. Each volunteer was asked to score each image on the following scale, taking into account the extensiveness and depth of depositions: 1 = pristine, no propolis; 2 = minimal deposition; 3 = moderate deposition; 4 = significant deposition; and 5 = heavy deposition.

We analyzed the data using linear mixed-effects models, which account for 1) the repeated measures of propolis deposition over time in the same colonies and 2) apiary-level effects. Thus, we used colony nested within apiary as random effects for our analysis. Given that the raw response variable is ordinal (integer scale from 1 to 5), with the same five volunteers scoring propolis levels in each colony on each sample date, we used the mean of the ordinal responses as our modeled response variable. Our models included as fixed effects the treatment (hive interior texturing method), the sample date (given that we expect increases in propolis deposition over time), and their interaction. We ran linear mixed-effects models using the 'lme4' package (Bates et al. 2015) for the



Fig. 2. Wooden hive wall interior modified with five parallel saw kerfs, 7 cm apart, cut 0.3 cm deep into the surface to texturize interiors to promote propolis deposition.

R statistical programming language (R Core Team 2017). We compared the hive box treatments in a pairwise fashion using post hoc Tukey HSD tests, with the ‘lsmeans’ package in R (Lenth 2016). We conducted extensive model validation via plotting; our data were normally distributed and there were no obvious relationships between fitted values and residuals. We concluded that our data meet the basic assumptions needed for analysis. A full reproducible report of the analysis (including the data analyzed), generated from Rmarkdown, is available in [Supplementary Material](#).

Results

Our mixed-effect model showed that hive interior treatment had a statistically significant effect on propolis deposition ($P = 0.0028$), as did sample date, with propolis hoarding increasing over time ($P = 5.184 \times 10^{-11}$). In addition, there was a significant hive box treatment \times sample date interaction ($P = 2.044 \times 10^{-05}$) (Fig. 4). Examining the pairwise comparisons between hive box treatments (Table 1), each of the texturing methods stimulated significantly more propolis hoarding compared with the nontextured controls, but none of the texturing methods was statistically distinguishable from another.

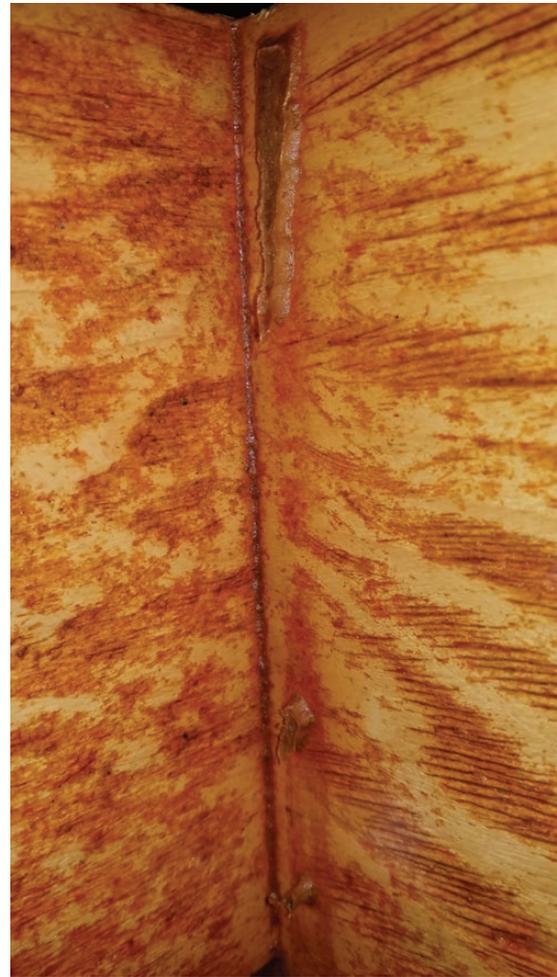


Fig. 3. Wooden hive wall interior roughened with a mechanized wire brush to texturize interiors to promote propolis deposition.

Discussion

Each of the three hive interior–texturizing treatments significantly increased propolis deposition compared with nontexturized controls; however, none of the three texturizing methods was significantly different from another texturizing method (Table 1).

The significant effect of sampling date shows that propolis deposition increased additively over time; however, there was also an interaction between sampling date and texturizing method. This interaction is apparent in Fig. 4 and is explained by the behavior of the saw kerf treatment. Propolis deposition in hives with parallel saw kerfs increased only marginally over time, actually decreasing in the middle two sampling dates before increasing above initial levels by the final sampling. This inconsistent performance by saw kerfs suggests that bigger sample size would have shown deposition with this treatment significantly lower than in the other texturizing methods. Compared with plastic propolis traps or roughened interior surface, the textural stimulus of saw kerfs is more finite; bees conceivably filled up the linear kerfs more quickly than the more complex surface irregularities of the other two treatments, thus canceling the hoarding stimulus prematurely (Fig. 2). It is possible that including more parallel kerfs could correct for this, but we anticipate structural compromise to hive parts with so many kerfs; on that point, the equipment manufacturer noted the risk of interior saw kerfs breaching to

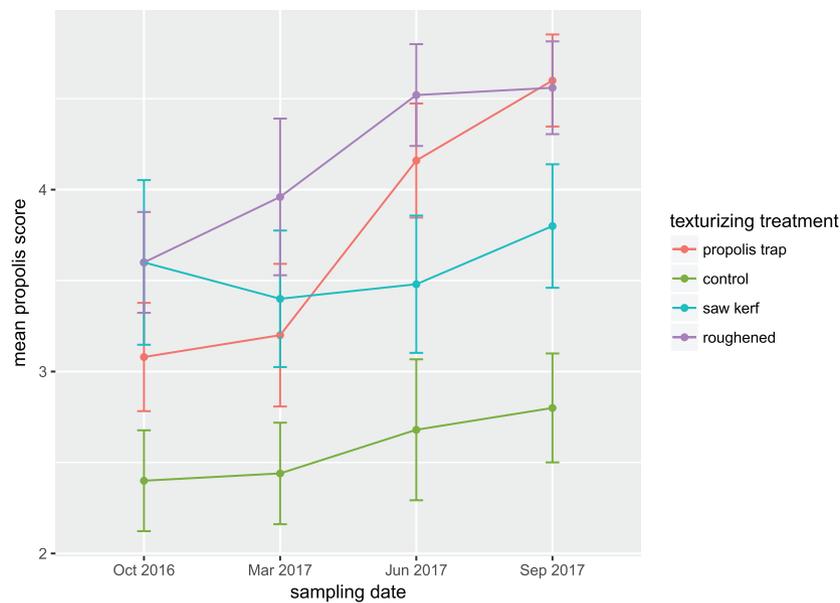


Fig. 4. Mean subjective scores for propolis deposition on hive interiors modified to provide bees different types of textural stimuli. Test bee colonies were set up August 2016 and scored at four successive sampling dates. Volunteer observers were asked to score digital photographs of hive interiors with the following scale of propolis deposition: 1 = pristine, no propolis; 2 = minimal deposition; 3 = moderate deposition; 4 = significant deposition; 5 = heavy deposition. There was a significant interaction between sampling date and texturizing method which was explained by the comparatively low rates of deposition exhibited by the saw kerf treatment.

Table 1. Pairwise contrasts between hive wall interior–texturizing treatments

Contrast	Estimate	SE	df	<i>t</i> ratio	<i>P</i>
propolis trap—control	1.18	0.2695985	12	4.377	0.0043
propolis trap—kerfs	0.19	0.2695985	12	0.705	0.8933
propolis trap—roughened	-0.40	0.2695985	12	-1.484	0.4760
Control—kerfs	-0.99	0.2695985	12	-3.672	0.0146
Control—roughened	-1.58	0.2695985	12	-5.861	0.0004
Kerfs—roughened	-0.59	0.2695985	12	-2.188	0.1816

the box exterior if the kerf was sawn directly opposite the recessed hand hold on the outside of the box.

We included saw kerfs as a treatment because it seemed a simple way to incorporate interior textural complexity directly into the manufacturing process. However, the sustained strong performance of roughened interior surfaces (Figs. 3 and 4) suggests a better optimization between propolis hoarding and manufacturing simplicity—the use of lumber that is left naturally rough, unplanned, on the interior side. Compared with plastic propolis traps stapled to interior walls (ca. 3 mm thick), we speculate that unplanned lumber has the added benefit of not subtracting from the bee space engineered into hive equipment by manufacturers to ensure easy insertion and removal of comb frames.

Although we did not include direct comparisons of bee health among our treatments, the benefits of increased in-hive propolis deposition have been convincingly shown by others. Our study suggests that relatively simple changes to the manufacturing process could incorporate textural complexity into hive designs with direct benefit for bee health.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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