A 3D Printed Pollen Trap for Bumble Bee (*Bombus*) Hive Entrances

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Abstract

To verify the plant sources from which bumble bees forage for pollen, individuals must be collected to remove their corbicular pollen loads for analysis. This has traditionally been done by netting foragers at nest entrances or on flowers, chilling the bees on ice, and then removing the pollen loads from the corbiculae with forceps or a brush. This method is time and labor intensive, may alter normal foraging behavior, and can result in stinging incidents for the worker performing the task. Pollen traps, such as those used on honey bee hives, collect pollen by dislodging corbicular pollen loads from the legs of workers as they pass through screens at the nest entrance. Traps can remove a large quantity of pollen from returning forager bees with minimal labor, yet to date no such trap is available for use with bumble bee colonies. Workers within a bumble bee colony can vary in size making size selection of entrances difficult to adapt this mechanism to commercially reared bumble bee hives. Using 3D printing design programs, we created a pollen trap that successfully removes the corbicular pollen loads from the legs of returning bumble bee foragers. This method significantly reduces the amount of time required by researchers to collect pollen from bumble bee foragers returning to the colony. We present the design, results of pollen removal efficiency tests, and suggest areas of modifications for investigators to adapt traps to a variety of bumble bee species or nest box designs.

Introduction

Bumble bees (*Bombus* spp.) are large robust insects that are found across the temperate, alpine, and arctic regions of the world[1]. They are important to plant communities and provide important pollination service for the agricultural crops that they visit[2]. Recent declines in the abundance and distribution of several species has brought their importance as pollinators to the forefront of public awareness[3]. Researchers have identified several stressors that are likely contributing to population declines including a lack of diverse and abundant floral resources on which bumble bees forage[4]. Identifying which plant species bumble bees forage from allows researchers and land managers to understand how bumble bees may be responding to changes in resource availability, competition, and anthropogenic disturbances[5,6].

Studies investigating the pollen foraging preferences of bumble bees are often conducted by researchers catching individual bees foraging at flowers, and then removing the corbicular pollen loads from specimens for further processing and identification[7,8,9,10]. While this method provides insight into how a species or an assemblage of bumble bee species utilizes the resources in an area[7], it is time intensive and potential differences in preferences among hives cannot be discerned without additional molecular analyses to identify colony of origin of the foraging bee[11].

For some studies of foraging dynamics, it is desired to conduct the studies at individual colonies; however, wild bumble bee nests are generally located underground or at ground level making them difficult to locate[12]. Commercially produced bumble bee hives provide researchers greater access and better experimental control and the removal of pollen off workers is still primarily conducted by capturing foragers as they return to the hive and manually removing their corbicular pollen loads[13,14]. The removal of pollen by hand from the corbicula of a bee is time intensive with a low hourly yield of pollen especially at hive entrances where the rate of returning pollen foragers may be low. Additionally, manually removing pollen from bees can result in stings from disturbed workers.

Pollen traps have been used for experimental removal of pollen from honey bees for decades[15]; yet, a passive method for removing pollen from bumble bees has not been developed. The primary obstacle in developing a mechanism to remove pollen from returning forager bumble bees is the large variation of worker sizes that exist in a bumble bee colony[16]. Honey bee pollen traps are effective largely because honey bee worker size does not vary much. Additionally, these traps require only minor manipulations after installation and don't require bees to be sacrificed[17]. This is achieved using screens or plastic surfaces that dislodge the pollen off of the hind legs of workers as they return to the hive. These traps remove only a portion of the pollen loads from returning foragers and the various designs of those result in varied efficiencies at pollen collection. As the pollen is removed from the bee legs, it falls through a screen and into a collection basin to which the bees have no access, so that the researcher can remove it with only minor disturbance to the hive.

The purpose of the present study is to adapt the techniques used for collecting pollen from honey bee hives and apply them to bumble bee nests using 3D printed structures and test the trap designs on colonies of *Bombus huntii*. The design process followed the assumptions that the traps should be inexpensive to produce, adaptable to a variety of bumble bee species, cause minimal harm or disturbance to the bees, and that the rate of pollen removal should exceed hand collection of pollen. Three-dimensional printing technology is versatile, easily accessible, and a cost-effective tool allowing researchers to replicate and modify objects for specific purposes[18]. The technique presented here instructs the user to build pollen traps and attach them onto commercially available bumble bee colonies. The traps are not designed to be use with wild colonies. These traps passively remove the corbicular pollen loads from the hind legs of pollen carrying bumble bees as they return to their nest boxes.

Protocol

1. Print pollen trap structures

1. Download the appropriate STL file for the nest box that bumble bees are nesting in (e.g., Biobest or Koppert style

hives, https://www.ars.usda.gov/pacific-west-area/logan-ut/pollinating-insect-biology-management-systematics-research/docs/pollen-traps/). The files are available to the public, free for download and modification by the end user.

2. Open the STL file in the printer program. Follow printer manufacturer directions to build the four trap components.
NOTE: Allow approximately 3 h for the trap body to print, 2 h for catch basin to print and 30 min each for the filter and trap closure insert to print. Trap body size is 6 cm x 3.8 cm x 7 cm.

2. Pollen trap assembly

1. Remove the support structures printed with the trap body and catch basin including those of the sieve structure of the trap body (**Figure 1**).

2. Use a 3/16 in. (0.476 cm) drill bit mounted in a hand drill to clear any plastic strands crossing the raised edges of the pollen filter that might hinder a bee from moving through the filter holes. Use a box cutting razor blade and sandpaper to even out any bumps or raised edges on the flat side of the pollen filter.

3. Place the pollen filter in the trap body by gently pushing the plastic filter through one side of the trap body. The filter will only fit in one way, as the left side of the trap has a larger opening to accommodate passage of the raised filter cones.

    1. If the side slits are too small for the pollen filter to slide through smoothly, scrape enough plastic away from the slit in the trap body with a razor or other tool that can remove small portions of plastic at a time. Ensure that the pollen filter fits securely in place with no more than a 2 mm gap between the pollen filter and trap body.

4. Attach the catch basin to the trap body by sliding the raised edges on the bottom of the trap body into the groove on the top of the catch basin. The catch basin should be positioned directly underneath the sieve region of the trap body (**Figure 1A–E**).

5. Make appropriate modifications by cutting or sanding the plastic to allow smooth placement and removal of the catch basin from the trap body. Placement of the catch basin will secure the pollen filter into place and it cannot be removed until the catch basin is removed, nor can the pollen filter be inserted while the catch basin is in place.
NOTE: If multiple hives are placed close to one another, providing each hive with a unique color combination of the trap body, catch basin and pollen filter structures in addition to deploying hives with varied orientation will help returning workers find their nests.

3. Bumble bee colony preparation

1. Stop pollen feeding 24–48 h before deploying colonies. This will cause workers to use up any stored pollen and stimulate them to leave the nest in search of pollen.

2. Prepare the hive trap body for installation by inserting a trap closure insert into the filter slot to prevent bees from escaping while installing the trap.

3. Working under red light to prevent the bees from flying, lift the plastic nest box out of the cardboard outer box.

4. Locate the plastic nest entrance at the front of the nest. There are two styles of entrance depending on the nest supplier: Koppert-style boxes (step 3.5) and Biobest-style boxes (step 3.6).

5. For Koppert-style hive entrances, mount the pollen trap onto the nest entrance by pulling up on the entrance tab until both entrance holes are open.
   1. Insert the two tubes of the pollen trap into the entrance holes, ensuring that the sieve of the pollen trap is on the bottom. Gently push down on the plastic entrance tab to secure the pollen trap in place.
6. To install the pollen trap into Biobest-style hive entrances, use a flat head screwdriver to gently pry the plastic entrance device from the nest box. Insert the pollen trap into the nest entrance holes until the pollen trap is firmly against the nest (**Figure 1E**).
   1. Secure the trap to the nest using tape or quick dry glue where the trap contacts the nest box if needed.
7. Return the plastic nest box to the cardboard box. Cardboard may need to be cut away to accommodate the pollen trap.

4. Deployment of nests

1. Place nest boxes into the study area. Provide cover to protect from precipitation and anchorage for wind as these can adversely affect the quality and quantity of pollen that is collected. Provide hives placed in greenhouses with adequate sun cover to reduce overheating.
2. Remove the trap closure insert from the trap body to allow bees to forage freely so that foragers can orient themselves with the surrounding area and the location of their nest. Orientation flight time should be complete in 24 h under normal conditions.

5. Pollen collection

1. To engage the trap, slide the pollen filter into the filter slot, ensuring that it is securely in place.
2. Install the catch basin by sliding it onto the trap body from the front until it is fully closed. If the catch basin is excessively loose or falls off the trap body, use a rubber band to secure it to the trap body.
3. Observe bees entering and exiting the pollen trap at first deployment to ensure the pollen filter holes are large enough to accommodate the bees.
   1. If workers are unable to pass through the pollen filter, remove the filter and then use drill bits larger than 3/16 in. (0.476 cm) to increase the holes sizes. Do so in a sequential manner, increasing hole diameter 1/32 in. (0.079 cm) each time, as holes that are too large will not collect any pollen.
   2. Once bees are able to pass through the filter, continue to observe the entrance to ensure pollen is being removed upon re-entry.

      NOTE: Bees should have some difficulty moving though the pollen filter holes, especially the first few times they pass through. If they pass through too easily, the pollen may not be dislodged from the corbiculae.
4. After the designated period of pollen collection, slide and remove catch basin from the trap body.
5. Process the pollen loads according to your experimental design.
6. Remove the pollen filter to allow workers to forage freely until the next period of pollen collection. The trap body may remain attached to the hive for the duration of the experiment.

   NOTE: Pollen traps may be engaged for as long as the researcher desires to collect pollen from a colony. However, deployment of pollen traps for over 24 h in a week may result in starvation of brood in a hive and retard colony development.

Representative Results

Eight different pollen filter designs were tested to determine their efficacy and efficiency at removing corbicular pollen loads from returning bumble bee workers. All designs were successful at removing at least of one corbicular pollen load from a returning forager. However, some were found to slow workers from leaving or entering the hive or failed to remove pollen loads (**Table 1).** Pollen traps with various filters were tested sequentially on 4 laboratory reared colonies of *B. huntii* Greene foraging on *Phacelia tanacetifolia* grown in greenhouses for a cumulative total of 138.5 h and 229 corbicular pollen loads (**Table 1**) collected over the 7-day period (3/2/16–3/8/16). Video cameras were placed in front of the nest entrances while pollen traps were engaged to record forager activity. Trap entrance design proceeded by trial and error over that period. Fifty-two hours of video observation and 142 corbicular pollen loads were collected during the test period (**Table 1**). Efficiencies were calculated by dividing the number of corbicular pollen loads collected by the number of observed pollen laden foragers that passed through a filter. Pollen filter design efficiencies ranged from 2–58.9% of full corbicular pollen loads removed. Corbicular pollen loads were removed from the legs and fell as cohesive pellets of pollen into the catch basin. Because of this tendency for corbicular loads to be removed as a pellet, partial corbicular pollen load removal was uncommon, but some partial loads may have been counted as full removal because we could not verify that some pollen remained in a corbicula after the bee entered the nest. Overall filter openings that were circular improved pollen collection and movement of workers into the nest environment. In addition, filter designs that had raised structures that extended away from the nest box also improved pollen removal from the hind legs of foragers. In a previous field study using an earlier filter design, the average weight of the pollen that was collected following 24 h of collection was 1.017 g over 11 hive-day collection periods. There was high variation (0.22–2.94 g per day) among the total mass of pollen collected from each nest. These values represent an expected mass range that pollen may be collected using this method. The final design in the download pollen trap print file is design number 8, a circular trap entrance with raised edges.

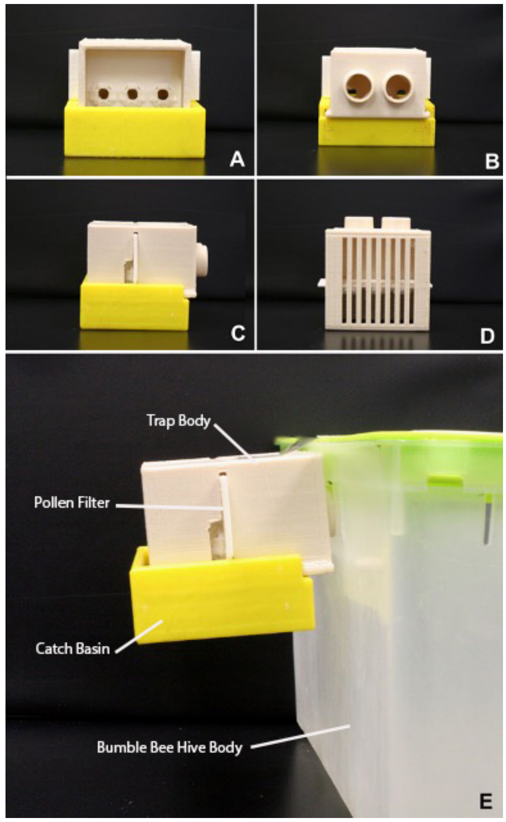

Figure 1: Pollen trap mounted to bumble bee hive. (**A**) Front view of pollen trap where workers land and travel across the sieve towards the pollen filter. (**B**) Posterior view of assembled pollen trap showing the grooved edges of the trap body that allow the catch basin to slide on and attach. (**C**) Side view of assembled pollen trap showing the pollen filter slit which allows the pollen filter to be placed into the trap body and secured by the catch basin. (**D**) Bottom view of trap body with pollen filter inserted, the sieve enables corbicular pollen loads to fall into the catch basin and restricts workers from accessing collected pollen. (**E**) Side view of assembled pollen trap attached to a nest box. Please click here to view a larger version of this figure.

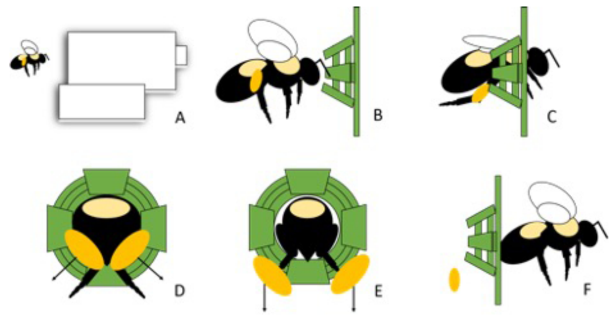

Figure 2: Mechanism by which corbicular pollen loads are removed from legs of workers. (**A**) Side view of a worker and its relative size to an assembled pollen trap. (**B**) Side view of worker approaching a pollen filter hole. (**C**) Side view of worker passing through a pollen filter hole forcing the corbicular pollen loads to contact the filter and ventral surface of the abdomen. (**D**) Posterior view of worker passing through a pollen filter hole forcing corbicular pollen loads to contact the filter and ventral surface of the abdomen. (**E**) Posterior view of worker passing through pollen filter hole once corbicular pollen loads have been stripped from its corbiculae and drop through the sieve and into the catch basin, and (**F**) side view of worker passing through pollen filter hole once corbicular pollen loads have been stripped from its corbiculae and drop through the sieve and into the catch basin.

		Cumulative Totals			Video Observation				
Design ID	Entrance Shape	Deployment (Hours)	Corbicular Pollen Loads Collected	Collection Rate (Pollen Loads/ hour)	Observation (Hours)	Corbicular Pollen Loads Collected	Pollen Foragers	Individual Efficiency*	Total Efficiency**
1	Diamond	1	1	1	1	1	9	0.11	5.56%
2	Diamond	3.5	2	0.57	3.5	2	50	0.04	2.00%
3	Square	4.5	2	0.44	4.5	2	2	1	50.00%
4	Circle	9.5	7	0.74	-	-	-	-	-
5	Circle	17.5	10	0.57	5.75	5	23	0.22	10.87%
6	Circle	18	36	2	13	35	54	0.65	32.41%
7	Circle	49.5	48	0.97	6.25	11	17	0.65	32.35%
8	Circle	35	123	3.51	18	86	73	1.18	58.90%
	Total	138.5	229	-	52	142	228	-	-
*Average number of corbicular pollen loads collected from returning pollen foragers.									
**Percentage of total corbicular pollen loads collected from returning foragers (Individual/2).									

Table 1: Summary table of the total deployment hours, corbicular pollen loads collected, collection rate along with the hours, number of pollen foragers, individual and total efficiency verified through video footage.

Discussion

Collection of pollen from bumble bee colony entrances can allow for a variety of ecological and agricultural studies. Identifying the floral sources from which bumble bees collect pollen provides valuable information and insight into the diversity of plants that contribute to a colony's overall diet[19]. Identifying the pollen source has implications for both agricultural production and studies of ecosystem services in wild lands[12, 20]. By gathering relatively large samples sizes of corbicular pollen loads, researchers can determine if bumble bees are foraging on the target crop for which they were deployed[21], other important constituents of the bumble bee diet[8], and preferred forage of specific species within an area[7]. Automating pollen collection from bumble bee colonies by using a pollen trap will allow for expanded studies of bumble bee foraging, nutrition and pesticide exposure.

Hand removal of pollen from bees, which has been used for the majority of studies investigating bumble bee pollen preferences, is time and labor intensive[10]. In contrast, passive collection of pollen using the pollen trap design collected over 200 corbicular pollen loads from four colonies over the course of a one-week period. Thus, this method allows researchers to collect pollen from multiple hives across different locations increasing both sampling effort and statistical robustness for future studies.

To develop and improve the pollen filter designs, video recording of the bumble bee workers passing through the trap mechanism was essential. We observed that when a bumble bee passes through a tight space, the hind legs extend behind and underneath its abdomen (**Figure 2C–E**). Observation of this behavior resulted in changes to the 3D printing design and trial and error testing of pollen filters (**Table 1**). Utilization of the video recordings for observation when deploying traps is recommended prior to initiation of the formal experiment to ensure that the traps are functioning properly and efficiently so that modifications can be made if necessary. To account for differences in body size both within a hive and among hives and the goals of the study, trap entrance size can be varied. We observed that adjusting the trap entrance to allow the larger foragers to exit and enter the hive provided minimal disruption to foraging activities and reasonable efficiency (>50%) removal of corbicular pollen loads. The 3D-printed plastic is easily modified with hand tools and print designs can be manipulated for specific projects or as box entrance designs change[18].

The limitations of this method are that pollen filter designs are unique to the species of bumble bee that is being sampled. This method uses a uniform size of entrance holes which, in theory, may restrict the larger individuals in the nest from foraging and smaller workers that forage may not be sampled; however, our design permitted large workers to pass through and we did not quantify efficiency based on body size. The design available for download is designed on the average size of *B. huntii* workers (3.22 mm thoracic width[22]) and thus that would need to be expanded as described for workers of *B. impatiens* (3.38 mm[23]) or *B. terrestris* (4.77 mm[24]). In one study using *B. terrestris*, larger individuals were noted to collect pollen more often than smaller workers[25]; however, a subsequent work found no correlation in worker size and the frequency of pollen foraging trips of the North American bumble bee *B. impatiens*[26,27,28]. Additionally, worker size may vary throughout the season[12], thus pollen filters should be inspected regularly to ensure that pollen collection is occurring efficiently. Understanding the species of interest and the specific research questions being addressed will be critical in assessing the utility of this trap design on a case-by-case basis.

Behavioral responses exhibited by returning foragers to the presence of engaged pollen traps were variable. These included: (i) workers acclimating to the additional effort needed to re-enter the nest environment, (ii) workers taking multiple attempts to pass through the filter, (iii) workers attempting to circumvent the filter opening and instead to pass through the trap body sieve, and (iv) workers avoiding the filter and switching to nectar gathering and transferring to nest bees through ventilation holes along the bottom of the nest box. Bumble bee foragers attempting to find alternative entrances to the hive had been observed in a previous study of this species[29] even when nest entrances are not blocked. While these responses were observed they were uncommon enough that we did not quantify the proportion of foragers who altered behavior due to the trap presence, except that most bees acclimated to the traps soon after trap deployment.

Future applications of this method include adapting existing designs for other commercially produced bumble bee species, particularly *B. terrestris* and *B. impatiens* which are primarily used for the pollination of greenhouse crops worldwide[19]. Use of these pollen traps on commercial hives in locations outside of their native range will allow researchers to determine what niche overlaps and competitive interactions may be occurring with native *Bombus* species[30, 31].

Disclosures

The authors have nothing to disclose.

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