Undergraduate Research Thesis

Granular Behavior of the Honey Bee Pollen Pellet and Associated Removal Mechanics

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1) Abstract

Honey bees collect and store pollen in the form of a pellet by packing the pollen grains together with regurgitated nectar. This research has indicated that the pollen pellet is a granulated suspension, i.e. a fluid that behaves largely as a solid due to capillary stresses on the surface of the granule. By alleviating these stresses, the granule can be melted (return to behaving as a liquid). Experiments that were performed involved melting the pollen pellet by bringing it into contact with the liquid from the suspension. It was found that the melting of the pellet is dependent on what type of pollen it consisted of. Pellets made from light yellow pollen melt when in contact with approximately 0.5 μL of 55% by mass sugar solution, while pellets made from dark yellow pollen do not melt with any amount of fluid contact, as determined from experimentation and imaging with a Scanning Electron Microscope (SEM). This phenomenon could be due to a differing amount of pollenkitt[1] (an adhesive substance secreted by pollen producing flowers) between the two types of pellets, or possibly due to the size difference between different pollen grains. Additionally, the mechanism by which honey bees remove pollen pellets depends on the speed of removal along with factors including the size, composition, and mass of the pellet. Pellets removed at the higher speed of 4.8 mm/s had an average peak force magnitude of 51 ± 33 mN, \ an average time for removal of 4.8 ± 2.2 sec, average peak energy of 0.16 ± 0.1 mJ, and average peak power of 0.78 ± 0.8 mW. Pellets tested at the lower speed of 1.0 mm/s had an average peak force magnitude of 24 ± 9.9 mN, an average time for removal of 3.6 ± 3.6 sec, average peak energy of 0.078 ± 0.03 mJ, and average peak power of 0.28 ± 0.5 mW. Future work will involve determining mechanisms of pollen pellet granulation through melting experiments and the creation of artificial pellets along with further collection of pollen pellet removal force measurements.

2) Introduction

In the field of biomimicry multiple disciplines, such as biology and physics, are often combined in order to study and understand the mechanics behind certain aspects of an animal system. Insects are particularly interesting to study due to the many unique abilities and mechanisms they possess. By combining the fields of entomology (a field which concerns the biology of insects) and mechanics (a physical science dealing with motions and forces) one can accurately describe the mechanism of a variety of insect structures, processes, and behaviors. Researchers in the field of biomimicry have created models describing various insect behaviors in the past, such as the behavior of fire ants forming rafts during floods and rainstorms by clinging

Figure 1. *A Honey Bee with a pollen pellet*[2]

to each other[3], and the behavior of water striders walking on the surface of water by taking advantage of surface tension[4]. Many of these kinds of behaviors rely on unconventional mechanisms that, if applied on a larger scale, could be novel solutions to many problems.

One mechanism that has not been well described is that by which honey bees collect and store pollen in pellets to be transported to their hive. The anatomy of honey bees is, however, well documented, as well as the field of fluid dynamics which comprehensively describes the properties of different types of fluids. The pollen pellet, as shown in Figure 1, consists of many pollen particles mixed with nectar. Past research has led to the idea that the pollen pellet may be a granular suspension, a type of fluid suspension where the particles are jammed, causing the suspension to behave more as a solid than a liquid. Jamming is caused by capillary stresses on the surface of the suspension that create force networks between the solid pollen particles. These forces can be relieved by Brownian motion or by contact with the pure liquid component of the suspension[5,6,7].

Additionally, the physiological mechanisms by which honey bees remove pollen pellets have not been well studied. The anatomy of the honey bee has been documented in great detail since the early 20th century, but the way that the anatomy interacts with pollen pellets has not[8]. Honey bees are capable of removing pollen pellets by exerting one or two pulsing forces onto their hind legs with their middle legs. This causes the pollen pellet to detach from the corbiculae without crumbling or separating. The precise forces and specific structures that allow for such a smooth removal are worth investigating.

If the properties of the pollen pellet could be determined along with a greater understanding of honey bee interactions with pollen pellets, a comprehensive model of the mechanism of pollen collection and storage could be created by combining entomological and fluid mechanical knowledge. Our research puts forth such a model to accurately describe the properties of the honey bee and the pollen pellet.

3) Materials and Methods

3.1) Volume Fraction

Before any experiments were attempted, the volume fraction of the pollen pellet needed to be determined. Pollen pellet samples were collected and prepared for imaging in a Phenom SEM by attaching a carbon tape sticker onto an SEM sample pin, placing a pollen pellet sample on the sticker, and sputtering a thin layer of gold over the pellet to ensure that its surface is conductive and will be visible in the SEM.

After obtaining multiple images from the SEM, image analysis was performed to measure the approximate volume fraction of each pellet. Using a software called Tracker, the average pollen grain diameter and the average distance between each grain in a defined area were determined. Different sections of 6 pollen pellet samples were imaged and the spacing between 10 particles was measured in each image. Using this information, the volume fraction of each sample was determined.

3.2) Melting Experiments

The first experiment that was conducted involved verifying that the pollen pellet is indeed a jammed suspension. This was done by employing standard techniques to alleviate the capillary stresses that maintain the structure of the pellet[7]. Brownian motion cannot be used to melt the

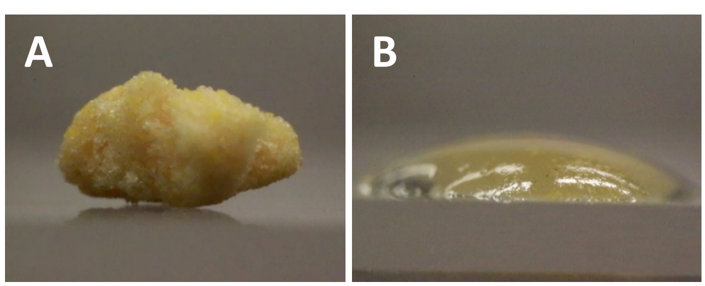

Figure 2. A: *A light-yellow pollen pellet before melting.*
B: *The same light-yellow pollen pellet after melting after 3 minutes and 1 µL of fluid was added.*

pollen pellet because its particles are too large for Brownian motion to affect the pellet. As a result, contact with the pure liquid was used for this test.

The experiments were filmed with a Canon 1D DSLR camera equipped with a macro lens. A 55% sugar solution was used as the pure liquid in the place of nectar. The camera began recording and, using a pipette, 0.5 μL droplets of the solution were dripped onto the pellet one after another, with enough time between drips to ensure that a majority of absorption had occurred. When enough solution was added that the pellet melted, the recording was stopped. By analyzing the video, the critical volume that caused melting can be determined and compared to literature on granular suspensions.

3.3) Removal Experiments

During the summer of 2018, another member on the research team filmed honey bees in the act of removing recently collected pollen pellets in their hive. These videos were analyzed to determine the speed and angles at which honey bees exert force on the pellet during removal. These videos showed that honey bees remove the pollen pellets in a series of 2-3 pushes with their middle legs. The speed of the pushes are slower in the first than the last pushes. For each hind leg of one individual bee, the first push occurred at an average speed of 0.77 mm/s and the second push at an average of 4.1 mm/s.

Following initial analysis of the honey bee videos, an apparatus was constructed to simulate and accurately measure removal forces on pollen pellet samples. The apparatus as seen in Figure 3A consisted of a servo motor controlled by an Arduino Uno that is programmed to rotate at a specific speed. A freely rotating double arm was attached to the servo and the rotation of the motor was translated to a linear motion by constraining the second arm in the structure shown in Figure

3A. This structure, consisting of laser cut plastic pieces, provided additional stability to the experiment, preventing the needle from deflecting when colliding with a pellet. A small needle was attached to the end of the arm that descends at a regular speed to remove pollen pellets. The

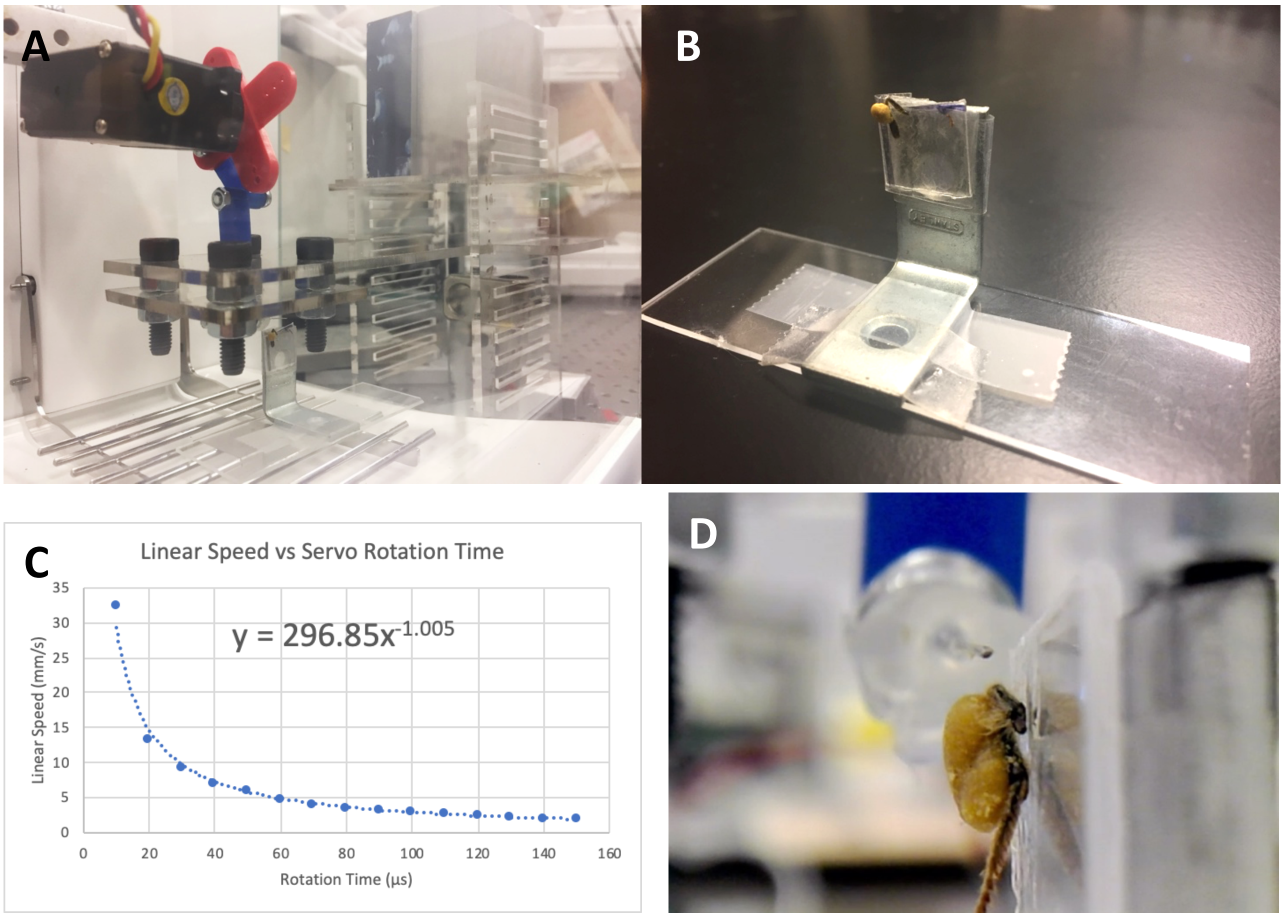

Figure 3. A: *Apparatus to measure removal force consisting of servo controlled by Arduino Uno and support structure.* ***B:*** *Pollen Pellet on leg glued to balance stand.* ***C:*** *Close-up USB Microscope image of pollen pellet during removal experiment.* ***D:*** *Calibration Curve representing relationship between Servo Rotation Delay and associated linear speed.*

pellets are glued, using UV curable glue (Loctite 4311) , by the leg to a stand to be placed on the mass balance as seen in Figure 3B. The Arduino was programmed to rotate a servo from 0° to 100° in a specific interval, or delay, of time. The shorter the delay, the faster the servo rotates from 0° to 100°. As a result, as lower delays are used, the servo rotates faster, and the translated linear speed becomes higher consequently. While the rotational speed maintains constant over the entire range of rotation, the translated linear speed changes sinusoidally, and is therefore not constant. Fortunately, during the initial descent the arm descends linearly and so the speed can be presumed constant. The pollen pellet was placed in this range of linear motion. Calibration tests were conducted to determine what Arduino delays resulted in what linear speeds, and these results can be seen in Figure 3C.

The experimental setup consisted of the pollen pellet still attached to the leg glued to a stand that was positioned beneath the needle of the apparatus. The stand was placed on top of a Mettler Toledo XS105 Dual Range Analytical Balance which acted as a force sensor. As the needle exerted force to remove the pellet, the mass balance recorded an increase in mass as a result of the increased force, which was captured on a computer using BalanceLink software. The mass was converted to force by multiplying by gravity, 9.81 m/s^2. A mass balance was used because force sensors designed to operate in the mN scale are difficult to find and expensive; most sensors available on the market are on the N or μN scale. The data collected by the mass balance over the period of removal was analyzed to determine the force exerted. An Andonstar USB microscope camera was used to obtain close up videos of the pellets being removed as well as to ensure proper positioning as seen in Figure 3D.

4) Results and Discussion

4.1) Volume Fraction

We calculate volume fraction by measuring the center-to center spacing of pollen particles in the pellet, and assuming this spacing is accurate for all sides of the particle, as shown in Figure 4. Using the equations

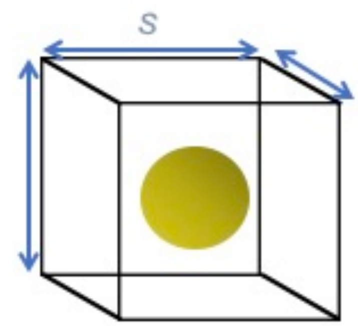

***Figure 4**. Volume Fraction Calculations*

$$\rho = \frac{1}{s^3} \tag{1}$$

$$N = \rho \times V_{pellet} \tag{2}$$

$$\phi = \frac{NV_{pollen}}{V_{pellet}} \tag{3}$$

where ρ is particle density, s is unit center-to-center spacing of the particles, V_{pellet} represents the volume of the whole pellet, V_{pollen} is the volume of an individual pollen grain, N is the total number of pollen particles in a pellet, and ϕ is the volume fraction, we calculate that the total number of pollen particles in a pellet range from 4 $\times 10^4$ to 16 $\times 10^4$, and the volume fraction ranges from 0.31 to 0.64 as seen in Table 1. This range of volume fraction is very high, with 0.64 being the largest possible volume fraction for the random close packing of spheres.

	Pellet 1	Pellet 2	Pellet 3	Pellet 4	Pellet 5	Pellet 6
s (mm)	0.03	0.03	0.03	0.03	0.04	0.03
ρ (#/mm^3)	55000	30000	52000	45000	22000	63000
N_{total}	16000	93000	16000	85000	41000	120000
V_{pellet} (mm^3)	3.00	3.04	3.09	1.88	1.88	1.88
V_{pollen} (mm^3)	1.16E-05	1.01E-05	1.22E-05	1.39E-05	2.32E-05	7.34E-06
Volume Fraction	0.64	0.31	0.63	0.63	0.51	0.46

Table 1*. Volume Fraction Calculations*

These calculations confirm that the pollen pellet acts as a jammed suspension. Guy et al. found that the critical stress, σ^*, at which shear thickening begins scales with particle size as $\sigma^* \sim d^{-2}$. Concentrated suspensions like the pollen pellet behave such that above a certain critical shear stress they will always be shear thickened. That is to say, they will only increase in viscosity with increasing shear stress. Because of the pollen pellet's large particle size and high volume fraction,

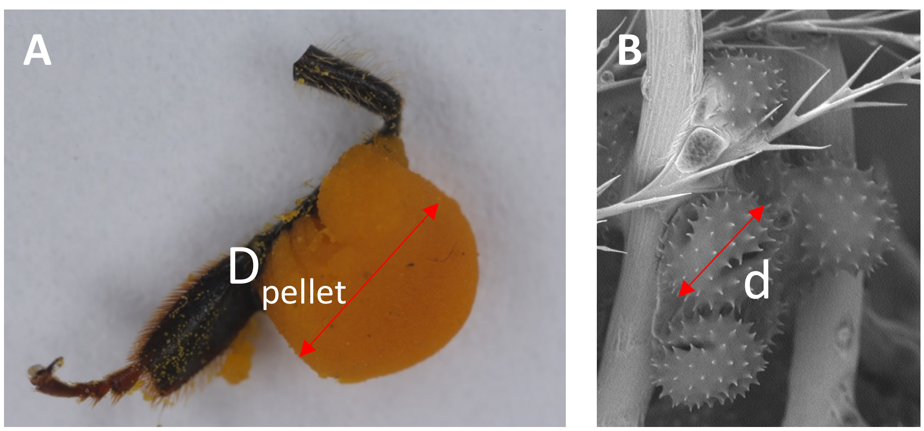

Figure 5. *Measuring pollen pellet and particle size for fluid property calculations.* ***A:*** *A pollen pellet attached to a honey bee leg with the diameter of the pellet indicated.* ***B:*** *An SEM image of an individual pollen grain in a pollen pellet, with the diameter of the pollen grain indicated.*

its viscosity increases rapidly with increasing shear stress, resulting in it taking the form of a jammed solid granule. Based on the findings of Guy et al. and using a pollen diameter of 20 µm, we calculated the critical shear stress for pollen grains to be 0.1 Pa. The shear stress experienced by pollen pellets due only to gravity is shown in Equation 5 below.

$$\sigma \sim \frac{mg}{D_{pellet}^2} \tag{5}$$

where σ is shear stress, $D_{pellet} \approx 2$ mm is the average diameter of a pollen pellet, $m \approx 0.002$ g is the mass of the pollen pellet, and g is acceleration due to gravity. Equation 4 gives a shear stress of approximately 10 Pa, which leads to the conclusion that the pellet is always shear thickened, forming it into a jammed state.

4.2) Melting Experiments

Melting experiments revealed that light yellow pollen pellets melted when enough sugar solution was dripped onto them. The amount of sugar solution required for the pellet to melt varies with the mass of the pollen pellet, with larger pellets requiring more solution, as seen in Figure 6.

Among the pollen pellets obtained, only light yellow ones melted, while dark yellow pollen pellets did not melt with any amount of sugar solution. SEM imaging of the pellet samples after being dripped with solution revealed that both pellets consisted of pollen grains without spikes,

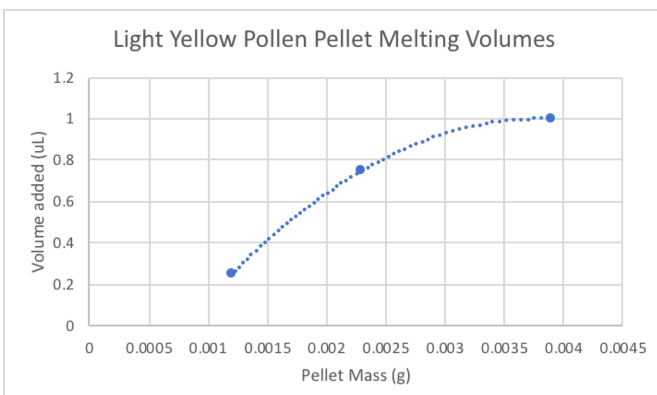

***Figure 6**. Required sugar solution volumes for melting in light yellow pollen pellets.*

although the light yellow pellets, with an average diameter of 81.6 µm, had approximately twice the diameter of pollen from dark yellow pellets, 43.7 µm, as seen in Figure 7.

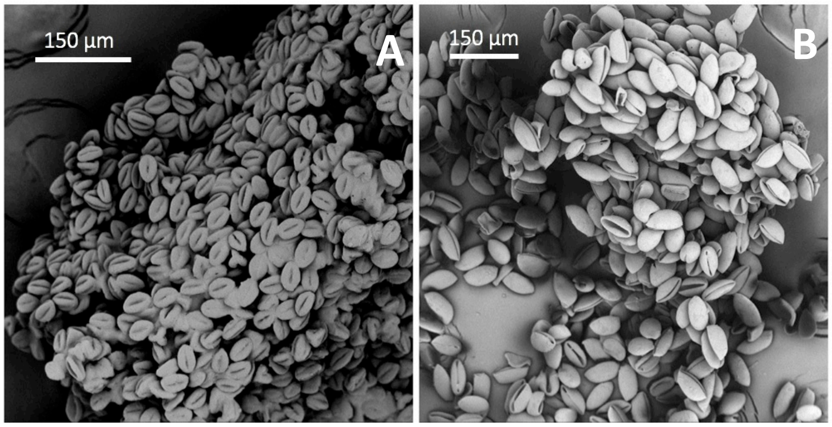

***Figure** 7. SEM images of pollen pellets after melting experiments. **A**: dark yellow pollen pellet which did not melt and had an average pollen grain diameter of 43.7 µm; **B:** light-yellow pollen pellet that did melt and had an average pollen grain diameter of 81.6 µm .*

It is possible that size difference between the two types of pollen grains contributes to dark yellow pellets not melting while light yellow pellets do. Another explanation could be pollenkitt, an oily substance produced by flowering plants that seems to serve a role in adhering pollen to pollinators[1]. Some species of flowers produce pollenkitt for a variety of benefits such as protecting the pollen from water, radiation, fungus, and bacteria, as well as making the pollen more attractive to pollinators and easier to transport[1]. Shin et al. have also found that the oily nature of pollenkitt keeps the aqueous nectar from evaporating from humidity and temperature changes[10]. Pollen pellets with larger pollen grains are known to consist of more pollenkitt than smaller grain pellets[1]. It is possible that the differing amounts of pollenkitt affect a pellet's ability to melt[9]. There may be a lower concentration of pollenkitt in dark yellow pollen pellets than light yellow ones, resulting in dark yellow pellets having a different mechanism forming the pellet, making it immune to melting through of fluid contact. The exact reason why some pollen pellets melt while others do not remains an open question and an area of future study.

4.3) Removal Experiments

For the first set of experiments with the supporting structures incorporated into the apparatus, an Arduino delay of 60 µs (equivalent to a linear speed of 4.9 mm/s) was used in order to obtain results of removal at faster speeds that could be compared with removal at actual speed. The results obtained from these experiments can be seen in Figure 8. The pellets tested had an average peak

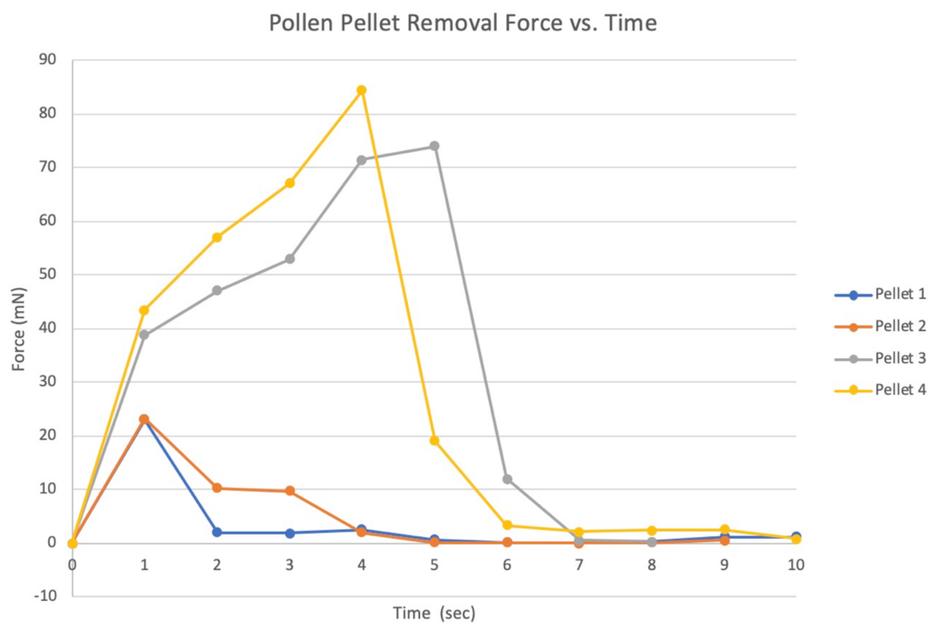

Figure 8. *Pollen Pellet Removal Force vs. Time at 4.9 mm/s*

force magnitude of 51 ± 33 mN, and an average time for removal of 4.8 ± 2.2 sec. The reason for these large standard deviations is possibly due to variations in the size, mass, shape, and type of pollen between each pellet. The same linear speed and experimental setup was used for all of them and all pollen pellets used were dark yellow in color. Additionally, the way that the corbiculae hairs attached to the pellets may have affected required force. Based on these results, average peak energy and power of removal were calculated for all four pellets as seen in Table 2. Average peak energy was calculated by multiplying peak force magnitude by the average length of a honey bee corbiculae (3.2 mm) , and found to be approximately 0.16 ± 0.1 mJ [11]. Average peak power was calculated by multiplying average peak energy by average removal duration, and was found to be approximately 0.78 ± 0.8 mW.

	Peak Force (mN)	Energy (mJ)	Duration (sec)	Power (mW)
Pellet 1	23.1	0.07	2	0.15
Pellet 2	23.2	0.07	4	0.30
Pellet 3	74	0.24	7	1.66
Pellet 4	84.5	0.27	6	1.62
Mean	**51.2**	**0.16**	**4.75**	**0.78**
Standard Deviation	**32.67**	**0.10**	**2.22**	**0.82**

Table 2*. 4.9 mm/s Removal Experiment Results and Calculations*

For the second set of experiments, an Arduino delay of 280 µs was used (equivalent to a linear speed of 1.0 mm/s) to closer emulate the speeds employed by honey bees, based on analysis of videos obtained at a bee hive of honey bees removing pollen pellets. The results of these experiments can be seen in Figure 9. The pellets tested had an average peak force magnitude of

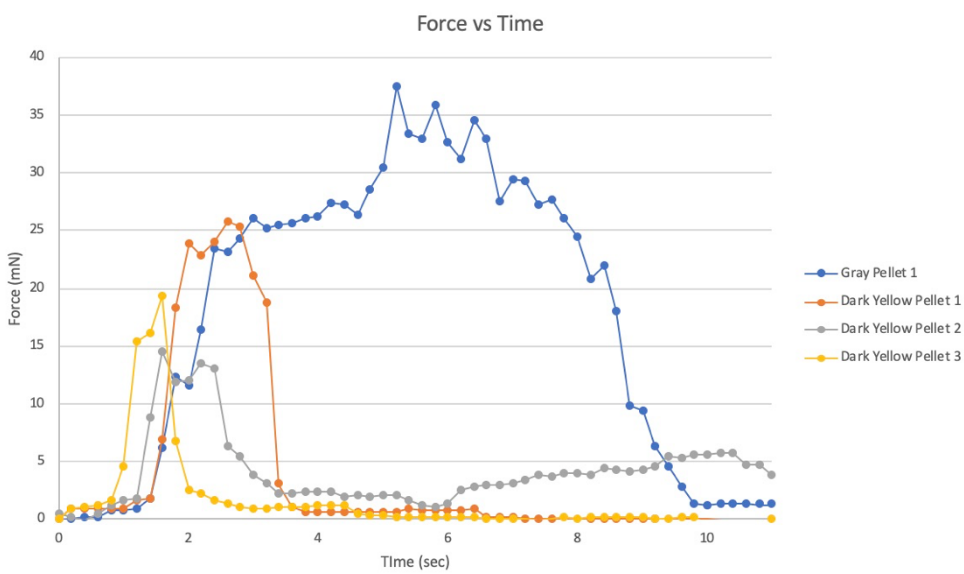

Figure 9*. Pollen Pellet Removal Force vs. Time at 1.0 mm/s*

24 ± 9.9 mN, and an average time for removal of 3.6 ± 3.6 sec. Again, average peak energy and power were calculated using the same equations as above for these lower speed trials as seen in

Table 3. Average peak energy was calculated to be 0.078 ± 0.03 mJ, and average peak power was calculated to be approximately 0.28 ± 0.5 mW.

	Peak Force (mN)	Energy (mJ)	Duration (sec)	Power (mW)
Gray Pellet 1	37.5	0.12	9	1.08
Dark Yellow Pellet 1	25.8	0.08	2	0.17
Dark Yellow Pellet 2	14.6	0.05	2	0.09
Dark Yellow Pellet 3	19.3	0.06	1.5	0.09
Mean	**24.3**	**0.08**	**3.625**	**0.28**
Standard Deviation	**9.93**	**0.03**	**3.59**	**0.48**

Table 3*. 1.0 mm/s Removal Experiment Results and Calculations*

The average peak energy and power were noticeably lower in the lower speed trials, with an average energy that is 2.0 times larger in the higher speed trial, and an average power that is 2.8 times larger in the higher speed trial, indicating that more energy is used in a full pollen pellet removal the higher the speed is used. Therefore, there may be an optimally low speed that removes the pellet quickly enough without consuming excessive amounts of energy.

Future work will be necessary to determine the full mechanics of pollen pellet removal. Variables such as pellet mass, pellet volume, type of pollen, time since collection, etc. may affect force required to remove the pellet. Currently, refinements are being made to the apparatus for a greater quantity of precise datasets can be obtained.

5) Conclusions

Pollen pellets consist of microscopic pollen particles at very high volume fractions, resulting in their formation as jammed solid granules. These granules can be melted by bringing them into contact with their base fluid, nectar. Certain pollen pellets melt easily when in contact with nectar, requiring more nectar to melt the larger the mass of the pellet. Other pollen pellets do not melt with any amount of contact with nectar, possibly as a result of differing particle sizes or the role of pollenkitt in the formation of pollen pellets. Future work will involve performing experiments to determine the role of the parameters in the behavior of jammed solid granules. Experiments will be performed to create artificial pollen pellets by combining nectar and pollen using a vortex mixer. Additionally, more work will need to be done to establish a comprehensive understanding of the mechanics of pollen pellet removal in honey bees. Preliminary research has shown that as the speed at which pellets are removed changes, the magnitude of force that is applied to the pellet as well as the time taken for the pellet to be removed changes considerably. Additionally, energy and power used to remove pellet seem to decrease as speed is decreased. A number of factors could affect these parameters including the mass of the pollen pellet, its size, the type of pollen used, the angle of application, etc. Future experiments will further investigate these factors to determine existing patterns in the mechanics of pollen pellet removal. The applications for this work include improved food stuffs, paints, pharmaceuticals, and machining processes[12,13]. Additionally, understanding the properties of the pollen pellet has applications in mitigating the harmful effects of declining honey bee populations through the development of artificial pollinators. Researchers have recently developed miniature robots that have the capability of performing the task of pollinators[14]. In order to develop fully efficient pollinators, it is important to know how the pollen is collected, transported, and removed so efficiently. This can be achieved through the fluid properties of the pollen pellet and the biomechanics of honey bees.

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