WV Pollen Project 2017

Bee Pollen Collaborator Report – July through October samples

Harold Davis,

I finished the analysis of your July through October pollen samples and wanted to send you a report on what I found. Specific details of the pollen extraction, treatment, and analysis procedure are mentioned below, followed by a summary of the contents of the samples.

I also want to thank you for the notes you took with each sample. It is always helpful to have a general idea of what is in bloom at the time each sample was taken, and this information also helps when comparing samples from different locations to understand the bloom season relative to the calendar date.

As beekeepers we have a limited knowledge of where our bees acquire their pollen, upon which they rely for their nutritional needs. I am hoping this study will give us an accurate and verifiable picture of this nutritional intake through the year in our wild and diverse West Virginia ecosystem, and support or improve our existing knowledge.

Extraction Procedure

To conduct the pollen study we first chose your July 14, July 29, August 10, August 24, September 12, and September 25 samples, as outlined in the project plan. From each sample we measured out 5 grams of pollen pellets to be sent to Texas A&M University for treatment. The remaining pollen in the vials you mailed to me is held for future reference.

Professor Bryant measures out two grams of pollen pellets into a sterile 15 ml screw-top centrifuge tube. This should contain nearly 200 pellets from samples of large pellets, and well over 200 pellets from samples containing normal-sized or smaller pellets. Provided that the pellets were well mixed, this amount should contain pellets from any pollen species comprising at least 0.5% to 1% of the pellets in the sample. This means that even if only 1% of the foragers in your colony were gathering pollen from a given flower type, pollen of that type should still show up in the sample. The pellets are then treated as follows:

1. Add glacial acetic acid (GAA) to the test tube to dehydrate the pollen, and then thoroughly mix the sample until all the pellets are dissolved.
2. Once dissolved, the samples are heated in a heating block at 80^{o}F for 5 minutes, stirred regularly, and then vortexed again to ensure all the pellets are dissolved properly and fully mixed.
3. Immediately after being vortexed, a sterile pipette is inserted into the middle of the mixture to extract about 4-5 ml of liquid.
4. The liquid is placed into a new, sterile 15 ml test tube, filled with GAA, and centrifuged at 3,500 rpm for 3 minutes before pouring off the GAA.

5. Add 8-9 ml of acetolysis, cook at 80°F for about 8 minutes, stirring regularly. The acetolysis chemical treatment (heating the sample in a mixture of sulfuric acid and acetic anhydride) is designed to remove lipids, waxes, and cytoplasm to allow easier identification of the pollen grains.
6. Remove the samples, centrifuge, and decant the acetolysis.
7. Wash the samples 3 times in distilled H_2O.
8. Stain the samples to create contrast for microscopic analysis and photography.
9. Rinse the sample in ETOH, centrifuge, then put into 2 ml vials and centrifuge again.
10. Pour off the ETOH, add 10-12 drops of glycerin, vortex the sample to mix the pollen with the glycerin
11. Seal the vials with an O-ring top and number.

Analysis procedure

When the treated samples have arrived back from Texas, we mix the tube at over 3,000 rpm in a vortex mixer until the sample is well mixed, then put a small drop of the glycerin containing the pollen grains onto a microscope slide, cover it with a cover-slip, and seal around the cover-slip with nail polish. Usually the solution needs to be diluted with more glycerin to make counting easier. The slide is numbered to match the pollen sample.

When dry, the slides are examined under 40x, 60x, and/or 100x (oil immersion) objectives to identify the pollen types present. Occasionally some frames may be photographed with a Nikon DS-Fi3 microscope camera. Time limitations have limited the ability to photograph pollen grains during this study.

Usually 400 pollen grains are counted and identified to establish a valid relative abundance of each pollen type in the sample. The general practice is to start at the lower right corner of the slide and work diagonally toward the center of the slide until 200 grains have been counted.

To the best of my knowledge, the recognized pollen percentage's classes for pollen pellet and bee bread would be the same as for honey:

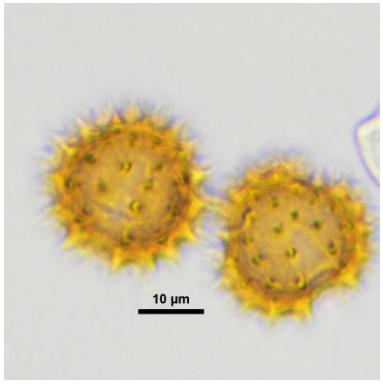
- Class A = >45%, called predominant pollen types
- Class B = 16-45%, called secondary pollen types
- Class C = 3-15%, called important minor pollen types
- Class D = <3%, called minor pollen types

Professor Bryant, palynologist at Texas A&M University has been of immense help in identification of pollen grains for this study. He describes the level of accuracy that can be made in pollen grain identification well: "In making quantitative counts, each pollen type is identified to the family, genus, or in some cases species level. Sometimes the pollen types within one plant family (such as the **Apiaceae** [umbels]; **Asteraceae** [composites]… **Poaceae** [grasses], **Rhamnaceae** [buckthorns], **Rosaceae** [rose family]… are diagnostic at the family level yet often many of their genera are not easily separated into specific types or species because of their morphological similarity with one another. In some other large plant families, such as **Fabaceae** (legumes), we are often able to identify some taxa to the generic level yet others in this family produce pollen types that are too similar to one another to distinguish at the

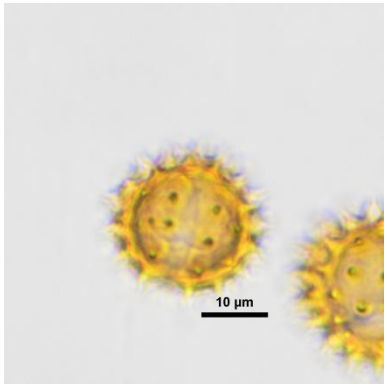
genus level without extensive reference collections and studies at levels of higher resolution scanning electron microscopy (SEM)."

One particularly unfortunate aspect of this study was the discovery that Snakeroot, Goldenrod, and Aster pollen grains are practically indistinguishable with light microscopy. I strongly desired to be able to report distinct values for these pollen types. Slight differences were not consistent enough to differentiate them in polyfloral samples, considering the variation even within one type as well as the presence of deformities. Furthermore the use of a single distinguishing factor such as a pore characteristic on otherwise identical grains cannot be used because, due to each grain's random position on the slide, the pore is usually not visible. Some slides contained a host of small Asteraceae type grains with every possible combination of variation in spine length, spine density, and grain size so that no lines of distinction could be drawn. On the bright side, in late-season samples after Snakeroot and Goldenrod had long faded, it could be safely assumed that the Asteraceae type present would be from Aster. Also, because Snakeroot pollen is white or light gray while Goldenrod is a deep golden color, a rough guess could be made based on the pollen pellet color of the frozen samples.

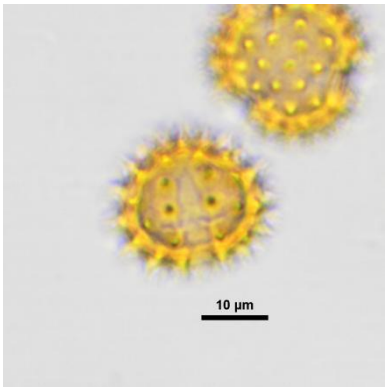
Below are some images of the pollen grains of Snakeroot, Goldenrod, and Aster showing their various features at a couple different angles.



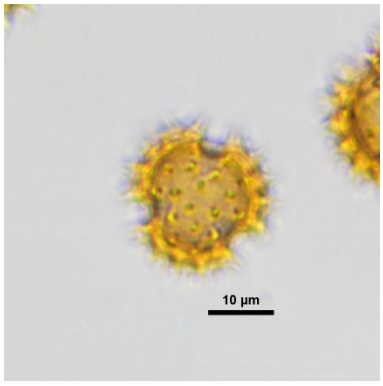
White Snakeroot (pore/furrow)



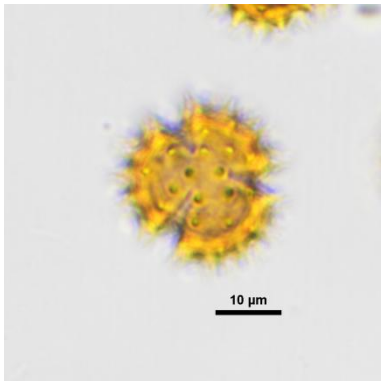
Tall Goldenrod (pore/furrow)



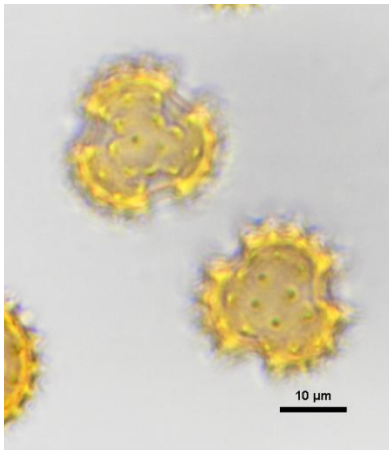
Late Aster (pore/furrow)



White Snakeroot (polar view)



Tall Goldenrod (polar view)



Late Aster (polar view)

Your Report

Special notes from each of your July through October samples are included below.

Following these comments is a **prevalence table** listing the pollen types found in your samples arranged in order of prevalence by percentage of samples (not by weight of pollen collected).

Next is the **table of pollen counts**, showing the counts of each pollen type in each sample with its percentage of the sample. To the right of the column titled Common Name is a count column and percentage column for each sample analyzed.

Next you will find a **bar graph** to help visualize the percentages of the different pollen types in each sample.

Finally I included a **line graph** showing pollen intake through the year in pounds.

July 14 (HD 15-07-14)

You noted that plants in bloom when your July 14 sample was collected included White Clover, Birdsfoot Trefoil, and Sourwood. I found the sample to contain 48.25% clover ("sweet clover" phenotype which also includes White Ladino clover), 31.25% chickory/lettuce type, 16.75% chestnut/tanoak type, 2.5% Teasel, and 0.25% each Plantain, Virginia Creeper type, Cornflower type, Thistle, and an unidentified grain.

The appearance of pollen grains of the Chicory and wild Lettuce type make me curious as to exactly which species the bees might be visiting. It is uncommon to see honey bees on Chicory and Wild Lettuce, yet these pollen grains appeared frequently in many samples throughout July and October. They are very similar to Dandelion pollen, which can bloom here and there throughout the summer due to mowing.

The small distinctive "Chestnut / Tanoak" pollen grains make me strongly suspect some ornamental Tanoak trees in the vicinity. It would seem a bit late for Chestnuts to still be blooming in the middle of July, whereas Tanoak are known to bloom in July and August.

Clover and Chestnut pollen are known to contain levels of protein sufficient for colony growth. I do not know if Tanoak pollen is similar to Chestnut. The document showing Chestnut pollen's protein level at "24%" was not adjusted for what the bees are able to obtain due to its specific amino acid profile. A deficiency of one or more of the essential amino acids can be a limiting factor in the amount of protein the bees are able to obtain from the pollen. ***If*** the ratio is not correctly balanced, the bees may not be able to use the full 24% protein from Chestnut. Another suspicion is that the Chicory/Wild Lettuce pollen might have a similar protein content as Dandelion would have, which is 15%. This is low, however it would be sought by bees if it contains specific amino acids that are lacking in the other pollen types available.

July 29 (HD 15-07-29)

You noted that plants in bloom when your July 29 sample was collected included white clover and an unknown 2nd color. I found the sample to contain 58.75% Chenopodium/Amaranth type (possibly Lambsquarters), 27.25% Chestnut/Tanoak type, 5.5% Chicory/Wild Lettuce type, 3% Ironweed, 2.75% Teasel, 1.75% Clover, 0.75% Sumac, and 0.25% unidentified.

I was actually surprised at the absence of sumac pollen which dominates most late July samples. The dominance of the Chenopodium/Amaranth type is interesting. I have seen bees vigorously working Lambsquarters *(Chenopodium album)* which leads me to suspect that this could be the source. It is interesting how little pollen the bees were obtaining from clover.

It would be worthwhile to find the protein content of plants in the Amaranthaceae family as this can sometimes be a major food source. See notes on the July 14 sample for comments on Chestnut and Chicory/Lettuce pollen.

August 10 (HD 15-08-10)

You noted that plants in bloom when your August 10 sample was collected included Wingstem and Early Goldenrod while White Clover was ending. I found the sample to contain 30% Virgin's Bower, 21.5% Wingstem/Sunflower type, 14% Elephant's Foot, 11.75% Chestnut/Tanoak, 8.25% Chenopodium/Amaranth type, 6.5% Asteraceae (possibly Goldenrod or Eupatorium types), 2.5% Clover, 2.25% Ragweed, 1.5% Plantain, 1% Chrysanthemum, and 0.75% Wild Carrot.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a single Thistle grain, likely from Tall Common Thistle, but not enough to amount to any appreciable percentage of the sample.

This was the only pollen sample analyzed in the project in which Virgin's Bower made up a greater percentage of the pollen than any other type. The 14% Elephant's Food does not surprise me, as I have seen bees very active on this plant many times, gathering pollen.

I know of no way at present to distinguish between Wingstem pollen and Sunflower pollen with light microscopy. Given the relative abundance of Wingstem in our region and the insignificance of most other wild and cultivated sunflowers, it might be safe to assume this pollen came from Wingstem.

As for the 6.5% "Goldenrod type" I would personally be surprised if it came from Early Goldenrod. I have yet to see honey bees visit Early Goldenrod, let alone gather pollen from it. I have

seen honey bees visit other plants blooming in August that would carry the same type of pollen grain such as Boneset, and perhaps other Eupatorium species as well.

It might be worthwhile to find the protein and nutrient content of Virgin's Bower pollen. If Wingstem pollen has a similar nutrient profile as Sunflower pollen, it would be considered low in protein and not able to sustain bees as the sole source of pollen, although it could be an important part of a varied diet, possibly supplying some elements lacking in other sources. The bees were able to find a variety of pollen types on this date, including a small amount of "Tanoak" and Clover.

August 24 (HD 15-08-24)

You noted that plants in bloom when your August 24 sample was collected included Early Goldenrod, Wingstem, and Japanese Knotweed. I found the sample to contain 37% Wingstem, 29% Clematis, 19.25% Elephant's Foot, 4.75% Chestnut/Tanoak, 2.5% Clover, 2.25% Goldenrod/Eupatorium type, 2% Chenopodium/ Amaranth type, 1.5% Plantain, 1% Wild Carrot, 0.75% Ragweed, and 0.25% Chicory/Wild Lettuce. I did not find any Japanese Knotweed; like you said, there was none nearby.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found an Ironweed grain and one that looked like Red Clover, but not enough to amount to any appreciable percentage of the sample.

See comments on previous samples regarding Chicory, Tanoak, Amaranth, Elephant's Foot, Wingstem, and Early Goldenrod. It appears the Tanoak bloom is starting to fade.

Nearly 60% of the sample came from species within the Asteraceae family (Wingstem, Elephant's Foot, Goldenrod/Eupatorium, and Ragweed). Generally these types tend to contain less than the desired 25% protein for good honey bee nutrition. Ragweed pollen by itself is known to be unable to support a colony, however with a variety of pollen types from different plant families as seen in this sample, it is possible that the bees would be able to obtain what they need to survive. It is hard to tell what high quality pollen could remain from earlier in the year, stored in the hive as bee bread.

September 12 (HD 15-09-12)

You noted that plants in bloom when your September 12 sample was collected included late Wingstem, Japanese Knotweed, and Clover, while Goldenrod and Snakeroot were coming into bloom. I found the sample to contain 95.5% Goldenrod/Snakeroot type, 2% Wingstem, 1.5% Clover, 1.25% Mistflower / Knapweed, 1% Elephant's Foot, and 0.75% Unidentified types.

It is striking to see the sudden change in pollen gathering when Goldenrod and Snakeroot began to come into bloom. They appear to have forgotten everything else in favor of these pollen types. Total pollen intake per hive increased from the previous sample by over 50%. This is a good indication that honey bees find Fall Goldenrod and Snakeroot pollen attractive, or at least that it provides an abundant amount of pollen. Here is where Goldenrod and Snakeroot pollen differentiation would be nice to have. As I mentioned above, the two pollen types are very difficult to distinguish with light microscopy. We can get a good lead however based on the color of the pollen pellets in the sample, because Snakeroot pollen is whitish in color while Goldenrod pollen is a golden orange color (see pollen loads on the bees' legs in the images below). It is obvious from the image of the sample's pellets above that the Goldenrod dominates significantly.


Honey Bee on Tall Goldenrod


Honey Bee on White Snakeroot


Honey Bee on Fall Aster

The unidentified pollen types also obviously belong to the Asteraceae family but were distinctly different from the Goldenrod or Wingstem types.

The fall-blooming Goldenrod is Canada Goldenrod, also known as Tall Goldenrod. Research indicates that the protein level in Canada Goldenrod pollen has dropped by a third from 18% to 12%, apparently due to the matching increase in atmospheric carbon dioxide[1]. We do not know if other pollen types or Asteraceae types specifically have been affected the same way. 12% protein is about half the protein concentration required to sustain honey bee colonies. This carries significant implications if Goldenrod pollen accounts for nearly 100% of the bees pollen intake as they prepare for winter.

([1] Ziska LH, Pettis JS, Edwards J, Hancock JE, Tomecek MB, Clark A, Dukes JS, Loladze I, Polley HW. 2016 Rising Atmospheric CO_2 is reducing the protein concentration of a floral pollen source essential for North American bees. *Proc. R. Soc. B* **283:** 20160414. http://dx.doi.org/10.1098/rspb.2016.0414)

September 25 (HD 15-09-25)

You noted that plants in bloom when your September 25 sample was collected included Asters in full bloom, Goldenrod ending, a few Snakeroot plants still blooming, and sparse White Clover. I found the sample to contain 96.75% Goldenrod / Snakeroot /

Aster pollen, 2.75% Wingstem / Sunflower, 0.25% Thistle, and 0.25% unidentified Asteraceae type.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a few Clover grains and a Chicory/Wild Lettuce grain, but not enough to amount to any appreciable percentage of the sample.

To determine how much of the sample consists of Goldenrod, Snakeroot, or Aster, it is obvious that there are not many whitish pellets in the sample, which leaves us mainly with the possibilities of Goldenrod and Aster. There are many Aster species blooming more or less at the same time and can have different colors of pollen ranging from pale to yellow to orange, so differentiation between Goldenrod and Aster cannot be ascertained with any certainty by the color of the pellets. Your notes stated that Goldenrod was ending, Aster bloom was in full, and the overall amount of pollen collected was greatly reduced. I would guess that Aster pollen could be making up a significant part of this sample.

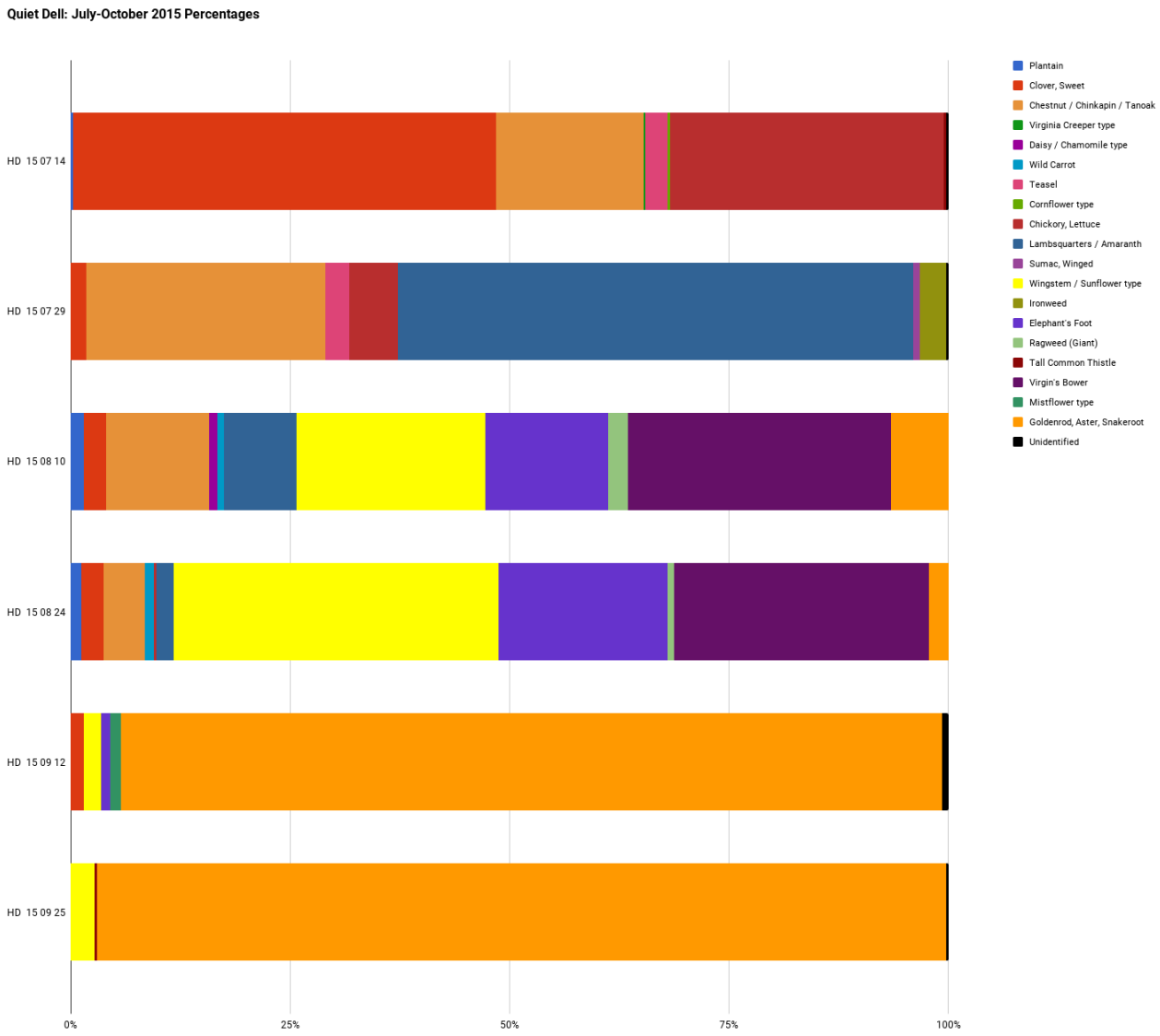
Prevalence Table: showing level of importance of Fall (July-October) Pollen Types in Your Neighborhood based on highest percentage found in samples analyzed:

Scientific Name	**Common Name**	**Highest % found**
Predominant Types		
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	97%
Chenopodium / Amaranth	Lambsquarters / Amaranth	59%
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	48%
Secondary Types		
Verbesina / Helianthus	Wingstem / Sunflower type	37%
Cichorium / Lactuca type	Chickory, Lettuce	31%
Clematis virginiana	Virgin's Bower	30%
Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	27%
Elephantopus	Elephant's Foot	19%
Important Minor Types		
Vernonia	Ironweed	3%
Dipsacus fullonums	Teasel	3%
Minor Types		
Ambrosia	Ragweed (Giant)	2%
Plantago	Plantain	2%
Conoclinium / Centaurea type	Mistflower / Knapweed type	1%
Chrysanthemum / Matricaria	Daisy / Chamomile type	1%
Apiaceae (i.e. Daucus)	Wild Carrot	1%
Rhus copallina	Sumac, Winged	1%
Cirsium altissimum	Tall Common Thistle	<1%
Parthenocussus	Virginia Creeper type	<1%
Centaurea cyanus type	Cornflower type	<1%

Table of Pollen Counts

Quiet Dell	2015	July				August				September			
Scientific Name	Common Name	HD 15 07 14		HD 15 07 29		HD 15 08 10		HD 15 08 24		HD 15 09 12		HD 15 09 25	
Plantago	Plantain	1	0%	0	0%	6	2%	5	1%	0	0%	0	0%
Melilotus & T. repens	Clover, Sweet & Ladino	193	48%	7	2%	10	3%	10	3%	6	2%	0	0%
Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	67	17%	109	27%	47	12%	19	5%	0	0%	0	0%
Parthenocussus	Virginia Creeper type	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Chrysanthemum / Matricaria	Daisy / Chamomile type	0	0%	0	0%	4	1%	0	0%	0	0%	0	0%
Apiaceae (i.e. Daucus)	Wild Carrot	0	0%	0	0%	3	1%	4	1%	0	0%	0	0%
Dipsacus fullonums	Teasel	10	3%	11	3%	0	0%	0	0%	0	0%	0	0%
Centaurea cyanus type	Cornflower type	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Cichorium / Lactuca type	Chickory, Lettuce	125	31%	22	6%	0	0%	1	0%	0	0%	0	0%
Chenopodium / Amaranth	Lambsquarters / Amaranth	0	0%	235	59%	33	8%	8	2%	0	0%	0	0%
Rhus copallina	Sumac, Winged	0	0%	3	1%	0	0%	0	0%	0	0%	0	0%
Verbesina / Helianthus	Wingstem / Sunflower type	0	0%	0	0%	86	22%	148	37%	8	2%	11	3%
Vernonia	Ironweed	0	0%	12	3%	0	0%	0	0%	0	0%	0	0%
Elephantopus	Elephant's Foot	0	0%	0	0%	56	14%	77	19%	4	1%	0	0%
Ambrosia	Ragweed (Giant)	0	0%	0	0%	9	2%	3	1%	0	0%	0	0%
Cirsium altissimum	Tall Common Thistle	1	0%	0	0%	0	0%	0	0%	0	0%	1	0%
Clematis virginiana	Virgin's Bower	0	0%	0	0%	120	30%	116	29%	0	0%	0	0%
Conoclinium / Centaurea	Mistflower / Knapweed	0	0%	0	0%	0	0%	0	0%	5	1%	0	0%
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	0	0%	0	0%	26	7%	9	2%	374	94%	387	97%
	Unidentified	1	0%	1	0%	0	0%	0	0%	3	1%	1	0%
		400	100%	400	100%	400	100%	400	100%	400	100%	400	100%

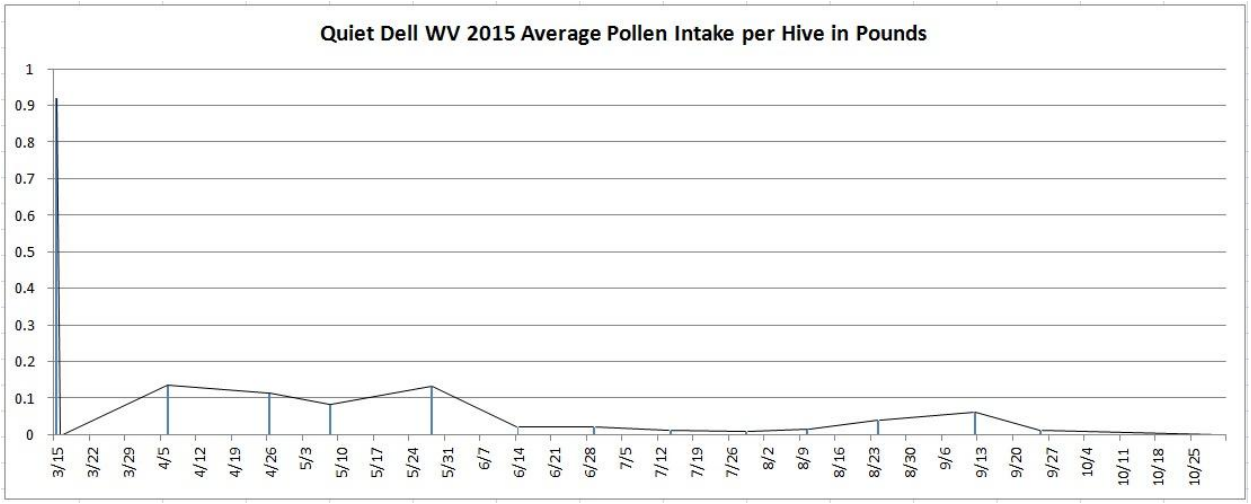
The information in the chart above is presented visually in the graph below. Each sample is listed on the vertical axis on the left. The percentages of each pollen type are listed along the horizontal axis in each bar. A separate color indentifies each pollen type. The graph shows the emergence and fading of each pollen type from sample to sample.



Remember that the graph above shows the ***percentage*** of each pollen type in the sample, not the ***amount*** of any type of pollen that was collected. One bar, such as HD 15-09-12, may represent a large amount of pollen while another bar such as HD 15-09-25 may represent a much smaller total amount.

Below is a graph showing the amount of pollen brought in through the year by weight. It is important to remember that the pollen was collected on favorable foraging days, and as we have seen, these days can be scarce at times due to unfavorable weather. The normal pollen intake therefore may be lower than is implied by the connecting lines displayed in the graph. It is also possible that pollen collection could have spiked higher at points between collection dates.

The vertical axis below is weight in pounds. The highest mark is one pound, about the maximum that would ever be collected in one day in our area with this method of collection. The dates at the bottom show 1-week intervals while the vertical lines show points at which samples were taken and the amount of pollen collected in that sample. *(You can ignore the high vertical line on the left which was used to create a uniform chart between all the collaborators.)*



Following very little pollen collection in June and July, it was a relief to see pollen collection pick up again in August and September. When pollen income is low, bees may be found foraging on less desirable pollens just because it is the only option available. On the other hand, there could be a quality pollen source, only the plant's population is too sparse to meet the demands of the colony. A drastic increase in pollen income was seen in other locations corresponding to the onset of Shining Sumac bloom *(Rhus copallina)*. There appears to be no Shining Sumac bloom in your area, so the introduction of this species or other late summer blooming trees might be highly beneficial. I did not find fungus spores in the sample, which bees may forage in a pollen dearth.

Honey bees foraging on brown rot spores from peaches.

Although I would expect the major pollen types gathered in July to have a suitable protein content, the bees gathered very little total pollen at this time. Toward the end of the year it is possible that the level of nutrition obtained by the bees was less than ideal. Traditionally, fall has been the chosen season to collect pollen from bees for human consumption or for sale. However some beekeepers have noticed lower survivability from colonies from which pollen was harvested in the fall. Spring pollen collection might be less jeopardizing for the colony because of a greater abundance and variety of pollens to make up for what is taken.

I hope this summary gives you an idea about the composition of the honey bees' diet in the months of July through October. Should you have any questions or desire additional clarification of this report please let me know.

Sincerely

Michael Staddon

This material is based upon work supported by Sustainable Agriculture Research and Education in the National Institute of Food and Agriculture, U.S. Department of Agriculture, under Award No. 2014-38640-22161. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author and do not necessarily reflect the view of the U.S. Department of Agriculture.