WV Pollen Project 2017

Bee Pollen Collaborator Report – July through October samples

Mark Lilly,

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 4, July 17, July 25, August 6, August 18, September 5, September 13, and September 31 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:

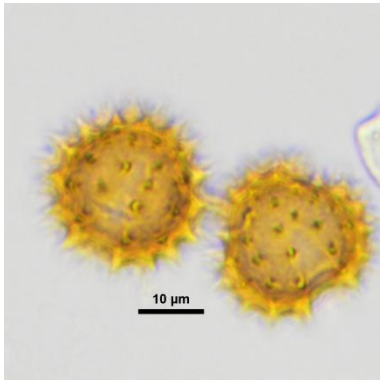
- Class A = >45%, called predominant pollen types
- Class B = 16-45%, called secondary pollen types
- Class C = 3-15%, called important minor pollen types
- Class D = <3%, called minor pollen types

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

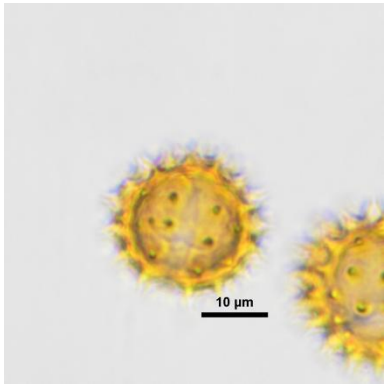
genus level without extensive reference collections and studies at levels of higher resolution scanning electron microscopy (SEM)."

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 or furrow width on otherwise identical grains cannot be used because, due to each grain's random position on the slide, these features are not always discernible. 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.

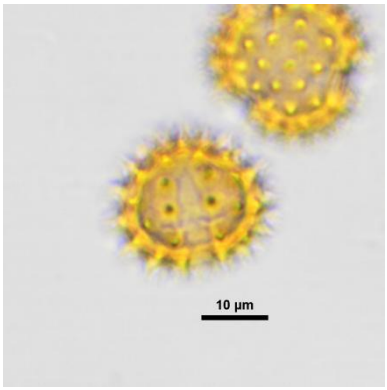
Below are some images of the pollen grains of Snakeroot, Goldenrod, and Aster showing their various features at a couple different angles.



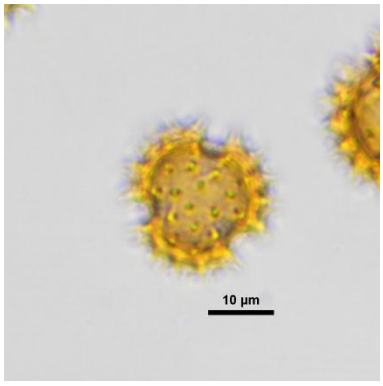
White Snakeroot (pore/furrow)



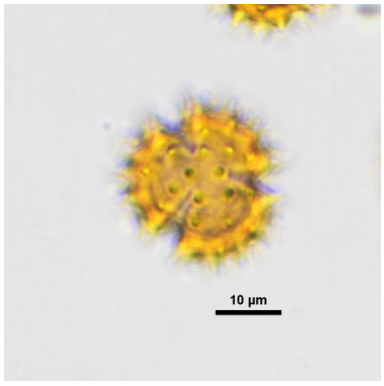
Tall Goldenrod (pore/furrow)



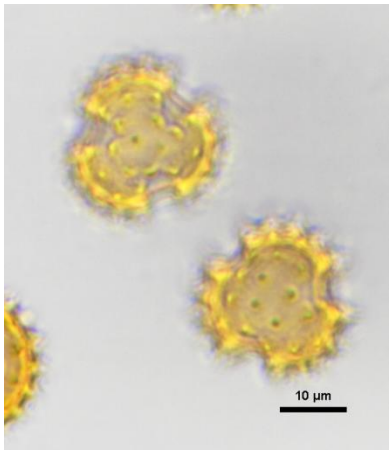
Late Aster (pore/furrow)



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 the sample (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 4 (ML 15-07-04)

You noted that plants in bloom when your July 4 sample was collected included Clover, Wild Chicory, and some Sourwood. I found the sample to contain 61.75% Thoroughwort type, 17.75% Red Clover, 6.75% clover ("sweet clover" phenotype, which includes White Ladino Clover), 6.75% Thistle, 4% Plantain, 2% Chicory / Lettuce type, 0.25% Wild Carrot, and 0.75% unidentified.

The "Thoroughwort" type pollen grains (*Asteraceae* family) could be one of numerous types of *Eupatorium* species such as *E. album*, *E. sessilifolium*, *E. serotinum*, *E. altissimum*. From what I could tell the grains in the sample were smaller than *Senecio* and I felt the spines were a bit too short to be from *Cosmos,* or *Rudbeckia* like Echinacea or Black-eyed Susan. Otherwise these were likely possibilities.

Asteraceae pollen types are generally known to contain lower levels of protein, not alone sufficient for colony health and growth. The low levels of total pollen being collected at this time of year could indicate that relatively little pollen is available, causing them to choose less desirable types. It would be very interesting to find out for certain which species provided the "Thoroughwort" type pollen in this sample

July 17 (ML 15-07-17)

You noted that clover was in bloom when your July 17 sample was collected. I found the sample to contain 30.75% clover ("sweet clover" phenotype, which includes White Ladino Clover), 25.75% Plantain, 16.75% Clematis, 13.75% Chicory/Wild Lettuce type, 5.5% Thoroughwort type, 4% Grass, 1.25% Cornflower type, 0.5% Corn, 0.5% Lambsquarters type, 0.5% Ironweed, 0.25% Thistle, 0.25% Virginia Creeper type, and 0.25% unidentified.

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. 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.

Total pollen collection at this time is still low. Clover pollen has a good level of protein to support bee colonies; apparently it was in short supply.

July 25 (ML 15-07-25)

You noted that Clover was still in bloom when your July 25 sample was collected, with Ironweed starting to bloom. I found the sample to contain 39.25% clover ("sweet clover" phenotype, which includes White Ladino Clover), 24% Plantain, 19% Clematis, 8.5% Chicory / Wild Lettuce type, 2.75% Thoroughwort, 2% Grass, 1.25% Virginia Creeper, 1% Dock, 0.75% Corn, 0.75% Cornflower, 0.25% Basil (or possibly a deformed Smartweed), 0.25% Chenopodium/Amaranth type (possibly Lambsquarters), and 0.25% Ironweed.

I was actually surprised at the absence of sumac pollen which dominates many late July samples and can be associated with a dramatic increase in total pollen intake. Total pollen intake remained low at this point in your area in 2015, but clover provides a good protein content, and together with the other types should help provide a balanced amino acid profile. It would be worthwhile to find out the protein content of Plantain pollen, especially the English (Buckhorn) Plantain which accounted for most of the 24%. About 2/5 are of the Broadleaf Plantain phenotype.

August 6 (HD 15-08-06)

You noted that plants in bloom when your August 6 sample was collected included Ironweed, Fall Asters, Joe Pye, and Clover. I found the sample to contain 39% Ragweed, 16.5% Goldenrod/Snakeroot/Aster type, 12% Clover ("sweet clover" phenotype, which includes White Ladino Clover), 11% Plantain, 10% Jewelweed, 4% Chicory / Wild Lettuce type, 2.25% Wild Carrot, 2% Clematis, 0.75% Grass, 0.5% Magnolia type, 0.25% Lambsquarters type, 0.25% Virginia Creeper, 0.25% Cornflower, 0.25% Ironweed, and 1% unidentified.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting. I found a Thistle grain, likely from Tall Common Thistle, a grain of Corn pollen, Red

Clover pollen, an unusual Sunflower type, as well as several large grains with an interesting almost striate surface, but not enough to amount to any appreciable percentage of the sample.

The ragweed pollen could be from Giant ragweed *(Ambrosia trifida)*, on which I have observed honey bees very active.

As for the 16.5% "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. It could be from the Asters you mentioned, or even Boneset or other Eupatorium species.

The few "Magnolia" pollen grains in this sample are a little mysterious. Not only in the samples from this project, but also in samples from other studies in the past, Magnolia type pollen grains have been identified in late-season pollen samples, far outside of Magnolia bloom time. The best alternative based on my references would be Yucca pollen, although not as close a match as Magnolia.

The total pollen intake was still low on this date. Ragweed pollen is known for its inability to support colony growth on its own, and may be gathered only when better sources are not available. It is hard to tell what high quality pollen could remain from earlier in the year, stored in the hive as bee bread.

August 18 (ML 15-08-18)

You noted that plants in bloom when your August 18 sample was collected included Ironweed, Poe-Pye, Aster, and Goldenrod starting. I found the sample to contain 73.5% Goldenrod / Snakeroot / Aster pollen, 20.75% Red Clover, 2.25% Clover ("sweet clover" phenotype, which includes White Ladino Clover), 2% Wild Carrot, 1% Chicory / Wild Lettuce type, 0.25% Ragweed, and 0.25% unidentified.

Although the volume of pollen the bees collected on this date is still low, it appears they are decidedly moving toward the Aster and Goldenrod sources even though the Goldenrod is just beginning.

September 5 (ML 15-09-05)

You noted that plants in bloom when your September 5 sample was collected included late Clover, Knotweed, Asters, and Goldenrod. I found the sample to contain 53% Ragweed, 21.75% Goldenrod / Aster / Snakeroot type, 9.75% Jewelweed, 4.75% Wild Carrot, 4.5% Plantain, 3.25% Chicory / Wild Lettuce type, 1.75% white sweet or ladino clover type, 0.5% Magnolia type, 0.25% Ironweed, and 0.5% unidentified.

Although the total pollen income seems to be increasing at this point it is interesting to see the return to Ragweed pollen. Around 75% of the last two samples consisted of pollen from species in the

Asteraceae family (Aster, Goldenrod, 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, it is possible that the bees would be able to obtain what they need to survive.

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). Ragweed pollen is yellow. Based on the image of this sample above, the yellow is probably Ragweed, the orange is probably Goldenrod, and Snakeroot is apparently absent.


Honey Bee on Tall Goldenrod


Honey Bee on White Snakeroot


Honey Bee on Fall Aster

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]. I 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 13 (ML 15-09-13)

You noted that plants in bloom when your September 13 sample was collected included Goldenrod, Clover, and Knotwood. I found the sample to contain 59.5% Ragweed, 25.75% Goldenrod / Snakeroot / Aster pollen, 5% Clover ("sweet clover" phenotype, which includes White Ladino Clover), 5% Plantain, 1.5% Jewel Weed, 1.75% Wild Carrot, 0.5% Chicory / Wild Lettuce type, 0.5% Ironweed, 0.25% Sunflower type, and 0.25% Thistle.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a few unusual types that I was not able to take the time to identify, as they did not make up any appreciable percentage of the sample.

This appears to have been the height of the fall pollen flow, and the samples do not show any significant spike in pollen gathering from Sumac, Wingstem, or Goldenrod .

Once again, the Asteraceae types comprise over 85% of this sample, likely indicating lower than ideal protein supply, but it is hard to tell what high quality pollen could remain from earlier in the year, stored in the hive as bee bread.

September 31 (ML 15-09-31)

You noted that plants in bloom when your September 31 sample was collected included Clover, Ironweed, Wild Aster, and Knotweed. I found the sample to contain 47.5% Virginia Creeper type, 20.75% Plantain, 10.75% Clover ("sweet clover" phenotype which includes White Ladino Clover), 6.5% Chicory / Wild Lettuce type, 5.75% Ragweed, 4% Goldenrod / Aster type, 3.75% Wild Carrot, and 1% Clematis.

It looks like Ragweed is going out of bloom. The reappearance of the Virginia Creeper type really floors me. The possibilities include Virginia Creeper, False Virginia Creeper, and Boston Ivy. What would make one of these bloom so late I'm not sure. Otherwise there would have to be some other plant type with pollen grains practically identical to Parthenocissus. More than two thirds of the Plantain pollen matches English Plantain (Buckhorn) while the others look like Broadleaf Plantain, lacking distinct pore margins.

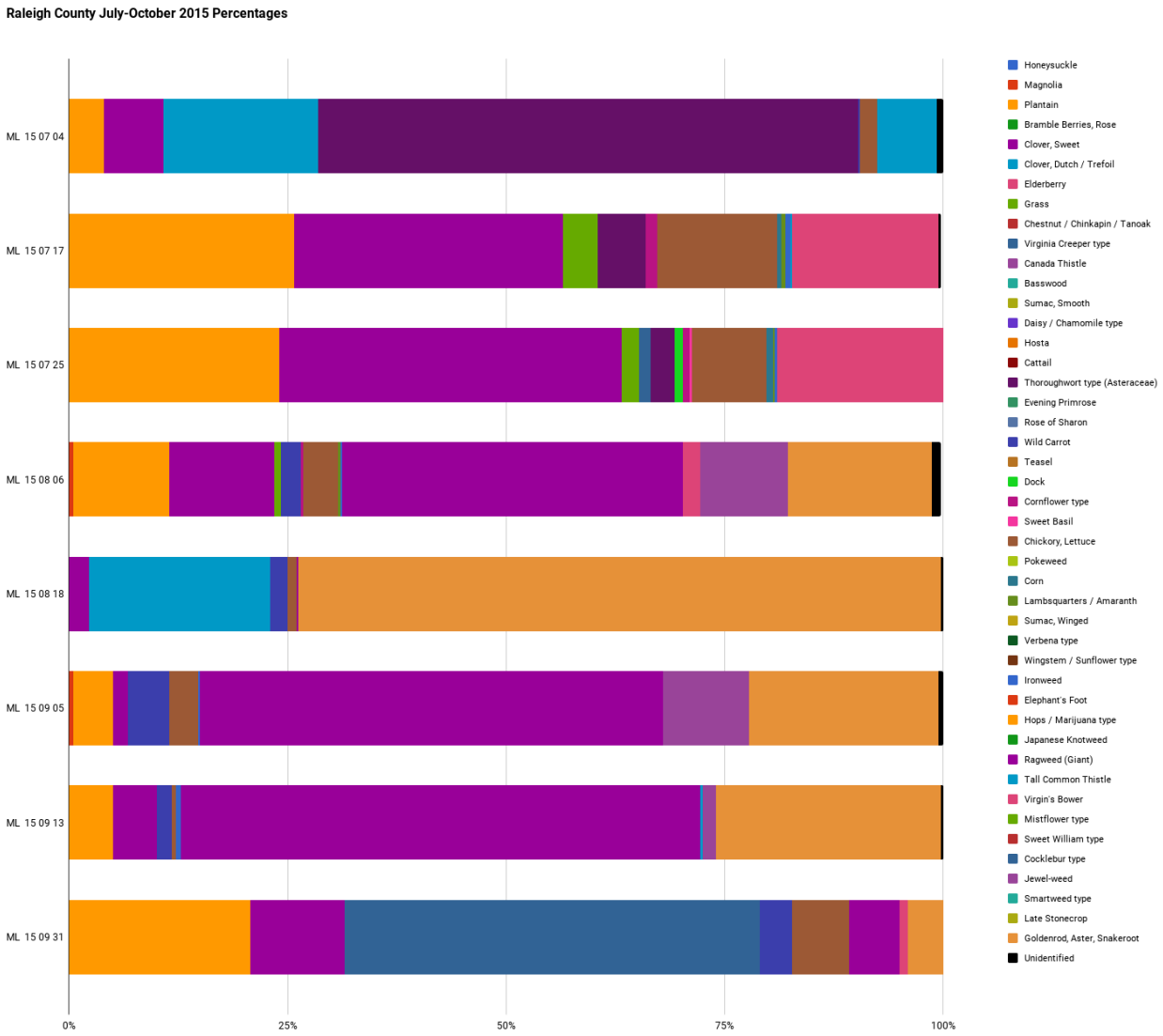
Prevalence Table: showing level of importance of Fall (July-October) Pollen Types in Your Neighborhood based on highest percentage found in samples analyzed:

Scientific Name	Common Name	Highest % found
Predominant Types		
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	74%
Eupatorium type (Asteraceae)	Thoroughwort type	62%
Ambrosia (likely A. trifida)	Ragweed (likely Giant)	60%
Parthenocussus	Virginia Creeper type	48%
Secondary Types		
Melilotus / Trifolium repens	Clover, Sweet & White Ladino	39%
Plantago	Plantain	26%
Trifolium	Red Clover, Crimson Clover	21%
Clematis virginiana	Virgin's Bower	19%
Important Minor Types		
Cichorium / Lactuca type	Chickory, Lettuce	14%
Impatiens	Jewel Weed (“Touch-Me-Not”)	10%
Cirsium altissimum	Tall Common Thistle	7%
Apiaceae (i.e. Daucus)	Wild Carrot	4.75%
Poaceae	Grass	4%
Minor Types		
Centaurea cyanus type	Cornflower type	1.25%
Rumex	Dock	1%
Zea mays	Corn	<1%
Magnolia	Magnolia	<1%
Chenopodium / Amaranth	Lambsquarters / Amaranth	<1%
Vernonia	Ironweed	<1%
Ocimum basilicum	Sweet Basil	<1%

Table of Pollen Counts

Raleigh County	2015	July						August				September					
Scientific Name	Common Name	ML 15 07 04		ML 15 07 17		ML 15 07 25		ML 15 08 06		ML 15 08 18		ML 15 09 05		ML 15 09 13		ML 15 09 31	
Magnolia	Magnolia	0	0%	0	0%	0	0%	2	1%	0	0%	2	1%	0	0%	0	0%
Plantago	Plantain	16	4%	103	26%	96	24%	44	11%	0	0%	18	5%	20	5%	83	21%
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	27	7%	123	31%	157	39%	48	12%	9	2%	7	2%	20	5%	43	11%
Trifolium pratense	Clover, Red	71	18%	0	0%	0	0%	0	0%	83	21%	0	0%	0	0%	0	0%
Poaceae	Grass	0	0%	16	4%	8	2%	3	1%	0	0%	0	0%	0	0%	0	0%
Parthenocussus	Virginia Creeper type	0	0%	1	0%	5	1%	1	0%	0	0%	0	0%	0	0%	190	48%
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	247	62%	22	6%	11	3%	0	0%	0	0%	0	0%	0	0%	0	0%
Apiaceae (i.e. Daucus)	Wild Carrot	1	0%	0	0%	0	0%	9	2%	8	2%	19	5%	7	2%	15	4%
Rumex	Dock	0	0%	0	0%	4	1%	0	0%	0	0%	0	0%	0	0%	0	0%
Centaurea cyanus type	Cornflower type	0	0%	5	1%	3	1%	1	0%	0	0%	0	0%	0	0%	0	0%
Ocimum basilicum	Sweet Basil	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Cichorium / Lactuca type	Chicory, Lettuce	8	2%	55	14%	34	9%	16	4%	4	1%	13	3%	2	1%	26	7%
Zea mays	Corn	0	0%	2	1%	3	1%	0	0%	0	0%	0	0%	0	0%	0	0%
Chenopodium / Amaranth	Lambsquarters / Amaranth	0	0%	2	1%	1	0%	1	0%	0	0%	0	0%	0	0%	0	0%
Vernonia	Ironweed	0	0%	2	1%	1	0%	1	0%	0	0%	1	0%	2	1%	0	0%
Ambrosia	Ragweed (Giant)	0	0%	0	0%	0	0%	156	39%	1	0%	212	53%	238	60%	23	6%
Cirsium altissimum	Tall Common Thistle	27	7%	1	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%
Clematis virginiana	Virgin's Bower	0	0%	67	17%	76	19%	8	2%	0	0%	0	0%	0	0%	4	1%
Impatiens capensis	Jewel-weed	0	0%	0	0%	0	0%	40	10%	0	0%	39	10%	6	2%	0	0%
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	0	0%	0	0%	0	0%	66	17%	294	74%	87	22%	103	26%	16	4%
	Unidentified	3	1%	1	0%	0	0%	4	1%	1	0%	2	1%	1	0%	0	0%
		400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%

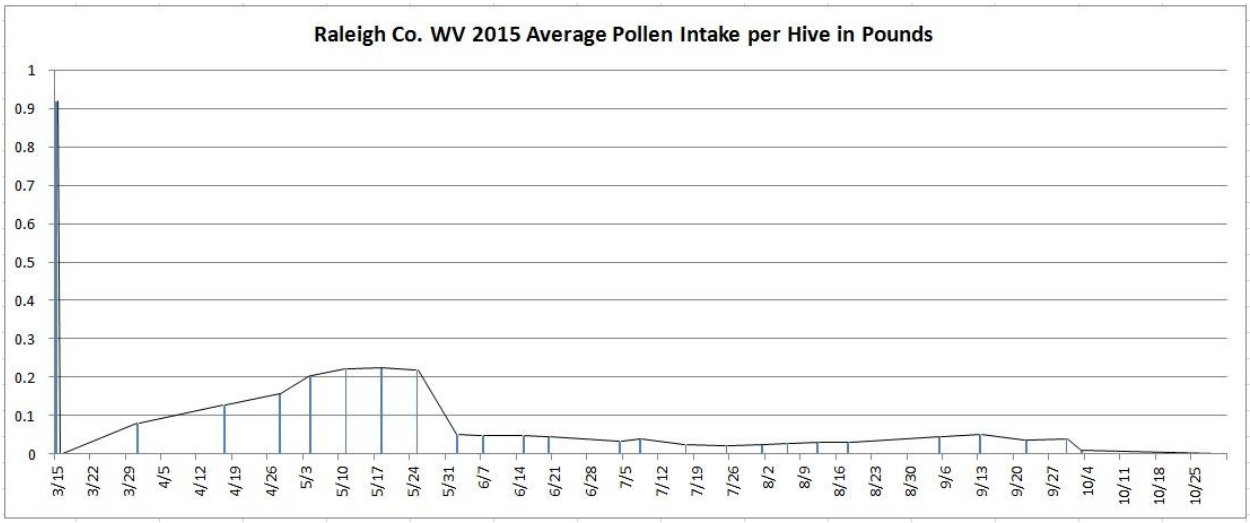
The information in the chart above is presented visually in the graph below. Each sample is listed on the vertical axis on the left. The percentages of each pollen type are listed along the horizontal axis in each bar. A separate color indentifies each pollen type. The graph shows the emergence and fading 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 ML 15-09-13, may represent a large amount of pollen while another bar such as ML 15-09-17 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.)*



It is interesting to see how much pollen was brought in through the spring compared to the fall. 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. There are other factors that can impact pollen gathering in addition to the bloom in the area, particularly colony health and weather.

Your samples were unique for their absence of any kind of Wingstem or Sunflower pollen. 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 meaningful Shining Sumac bloom in your area, so the introduction of this species or other late summer blooming trees might be beneficial. I did not find fungus spores in the sample, which bees may forage in a pollen dearth.

Mysterious aspects of you samples include "Magnolia" type pollen grains here and there and the Virginia Creeper type pollen showing up in late September.



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.