WV Pollen Project 2017

Bee Pollen Collaborator Report – July through October samples

Mike Blessing,

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 7, July 16, July 25, August 9, August 28, September 14, September 27, October 6, and October 14 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:

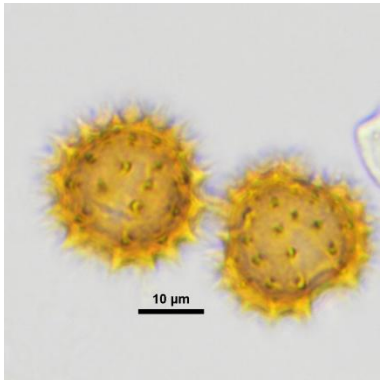
- Class A = >45%, called predominant pollen types
- Class B = 16-45%, called secondary pollen types
- Class C = 3-15%, called important minor pollen types
- Class D = <3%, called minor pollen types

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

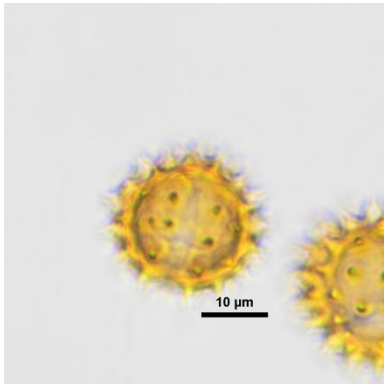
genus level without extensive reference collections and studies at levels of higher resolution scanning electron microscopy (SEM)."

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 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.

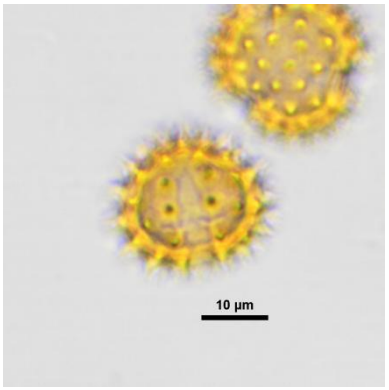
Below are some images of the pollen grains of Snakeroot, Goldenrod, and Aster showing their various features at a couple different angles.



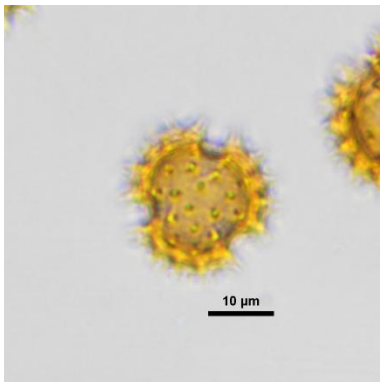
White Snakeroot (pore/furrow)



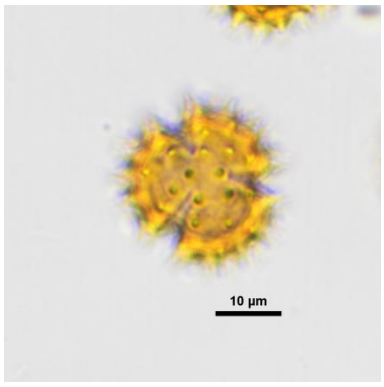
Tall Goldenrod (pore/furrow)



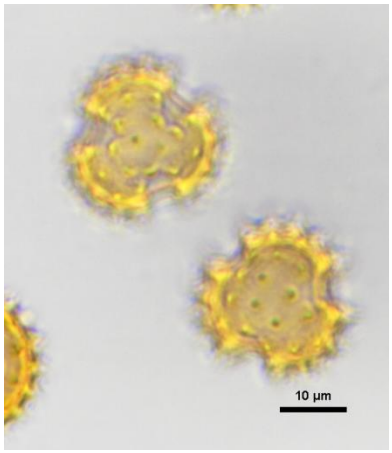
Late Aster (pore/furrow)



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 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 7 (MB 15-07-07)

You noted that plants in bloom when your July 7 sample was collected included White Clover, Rose of Sharon, Sourwood, and possibly Sunflowers. I found the sample to contain 62.75% Sumac, 27.5% Plantain, 6% Clover ("sweet clover" phenotype which includes White Ladino Clover), 1.75% Virgin's Bower, 1.5% Honeysuckle, 0.25% Chicory / Wild Lettuce type, and 0.25% Sweet Basil.

The amount of pollen brought in on this date was still low. It appears the Sumac trees, which would continue to supply a large percentage of the samples for the rest of the month, were just coming into bloom.

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 from many locations throughout the summer and fall. 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.

The Honeysuckle pollen is interesting, as it will continue to show up in low quantities through August, and again in October. Japanese Honeysuckle is the most likely suspicion, but I have had difficulty finding honey bees on this plant.

July 16 (MB 15-07-16)

You noted that plants in bloom when your July 16 sample was collected included white clover, sourwood, and various wildflowers, possibly sunflowers. I found the sample to contain 83.25% Sumac, 8.75% Plantain, 4.25% Clover ("sweet clover" phenotype which includes White Ladino Clover), 2.5% Bramble Berry type, 0.5% Virgin's Bower, 0.5% Honeysuckle, and 0.25% Corn.

The increasing volume of pollen and the high percentage of Sumac show this source to be significant for the bees. Plantain (especially English Plantain also known as Buckhorn) also keeps a significant presence in the samples, which I see from most other locations as well.

The quality of the bees overall forage at this time of year depends largely on Sumac. The vigor with which the bees gather this pollen is a good indication.

July 25 (MB 15-07-25)

You noted that plants in bloom when your July 25 sample was collected included some clover and various wildflowers, with lots of an unknown colorful orange color. I found the sample to contain 86% Sumac, 12.75% Plantain, 0.5% Clover ("sweet clover" phenotype which includes White Ladino Clover), 0.25% Virgin's Bower, 0.25% Honeysuckle, and 0.25% unidentified.

The unidentified grain looked mostly like a Red Clover grain. This appears to be the peak of the Shining Sumac bloom, and the bees brought in the largest amount of pollen for a single day as recorded all year. I only saw this dramatic spike in volume when Shining Sumac was in bloom. The availability of this pollen source could play a major role in bees' preparations for winter by raising the fat healthy population needed to raise the winter bees that take the colony through the cold months.

August 9 (MB 15-08-09)

You noted that not much was blooming, just various wildflowers, when your August 9 sample was collected. I found the sample to contain 44.5% Goldenrod / Aster / Snakeroot type, 34% Wingstem / Sunflower type, 9.25% Mistflower / Knapweed type, 2.5% Elephant's Foot, 2.5% Clover ("sweet clover" phenotype which includes White Ladino Clover), 2.5% Ironweed, 1.75% Hop / Hemp type, 0.75% Honeysuckle, 0.25% Ragweed, 0.25% Thistle, and 1.75% unidentified.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a few very large and striking pollen grains matching my images for Blue

Waxweed and Rough Buttonweed, but not enough to amount to any appreciable percentage of the sample.

In this sample we see some bloom types have ceased like the Sumac and Plantain, while typical fall bloomers begin such as Wingstem, Ironweed, Elephant's Foot and the Goldenrod / Snakeroot type.

The seven unidentified pollen grains looked like another sunflower type with much smaller and more numerous spines.

As for the 44.5% "Goldenrod type", I would personally be surprised if it came from Early Goldenrod. After many observations of Early Goldenrod patches near honey bee yards, I can count the honey bees from those flowers on one hand and have never seen a single bee gathering pollen. It is the later-blooming Canada Goldenrod (also known as Tall Goldenrod) that honey bees use. This generic Asteraceae "Goldenrod type" pollen that emerged in this sample continues to be prevalent for the rest of the year. You started noting Goldenrod bloom at the time of your September 6 samples. Not knowing the bloom season in your region personally, I have a hard time suggesting which Asteracea species is showing up in August – possibilities include Boneset, Joe-Pye-Weed, other Eupatorium species, and a rather early onset of Canada Goldenrod, and Asters, and perhaps other Asteraceae species. The orange color of the pellets would exclude Snakeroot, which is white. Because it was routinely found through the fall season I lumped them together under the title "Goldenrod / Snakeroot / Aster type".

August 28 (MB 15-08-28)

You noted that not much was blooming, just various wildflowers, when your August 9 sample was collected. I found the sample to contain 41.25% Wingstem / Sunflower type, 37.5% Goldenrod / Aster / Snakeroot type, 7.5% Mistflower / Knapweed type, 4% Elephant's Foot, 3.75% Clover ("sweet clover" phenotype which includes White Ladino Clover), 2.75% Ironweed, 2% Jewelweed, 0.75% Honeysuckle, 0.25% Thistle, and 0.25% Ragweed.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and again found a few of the grains that looked like Blue Waxweed or Rough Buttonweed, but not enough to amount to any appreciable percentage of the sample.

This was probably the only sample in the entire study in which the Wingstem / Sunflower pollen comprised the highest percentage of any type. 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. 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 Elephant's Foot is a plant few pay attention to, but which I have known bees to work quite vigorously for pollen in the fall.

Approximately 90% of the sample came from species within the Asteraceae family (Wingstem, Goldenrod / Snakeroot, Elephant's Foot, Mistflower / Knapweed, and Ragweed). Generally these types tend to contain less than the desired 25% protein for good honey bee nutrition. It is hard to tell what high quality pollen could remain from earlier in the year, stored in the hive as bee bread.

September 14 (MB 15-09-14)

You noted that plants in bloom when your September 14 sample was collected included Goldenrod and various wildflowers. I found the sample to contain 99.5% Goldenrod / Aster / Snakeroot type, 0.25% Elephant's Foot, and 0.25% Mistflower / Knapweed.

There is often a sudden change in pollen gathering when the fall blooming Goldenrod, Snakeroot, and Aster began to come into bloom, as these types overwhelm all the others in the samples. Here is where Goldenrod, Aster, and Snakeroot pollen differentiation would be nice to have. As I mentioned above, these pollen types are very difficult to distinguish with light microscopy. We can get a 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 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 27 (MB 15-09-27)

You noted that plants in bloom when your September 27 sample was collected included Goldenrod, Asters, and various wildflowers. I found the sample to contain 99.5% Goldenrod / Aster / Snakeroot type, 0.25% Elephant's Foot, and 0.25% Mistflower / Knapweed type.

The total amount of pollen being brought in at this time remains low. Sometimes there is an increase in pollen income during the fall Goldenrod flow, which your samples did not show.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and was surprised to find a Sumac pollen grain.

October 6 (MB 15-10-06)

You noted that plants in bloom when your October 6 sample was collected included Goldenrod, Asters, and various wildflowers. I found the sample to contain 97.25% Goldenrod / Aster / Snakeroot type, 2.5% Mistflower / Knapweed, and 0.25% Wingstem / Sunflower type.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a grass pollen grain, a thistle pollen grain, and one that was similar to Sunflower but with distinctly shorter and much more numerous spines. None were enough to amount to any appreciable percentage of the sample.

October 14 (MB 15-10-14)

You noted there were various wildflowers in bloom when your October 14 sample was collected. I found the sample to contain 99.75% Goldenrod / Aster / Snakeroot type and 0.25% Chicory / Wild Lettuce type.

Given that the Goldenrod flow is well over by now and Asters can continue to bloom much later than Goldenrod, I think it is fairly safe to assume that the majority of this pollen is from Asters.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a Honeysuckle pollen grain, but nothing enough to amount to any appreciable percentage of the sample. The Honeysuckle pollen grain definitely seemed out of character for October, however I found honeysuckle grains in other samples from other locations as well, so it is not just a fluke. Japanese honeysuckle vine is supposed to be able to continue blooming late into the year, although I do not know honey bees to visit it. Honey bees are evidently very good at finding whatever is available!

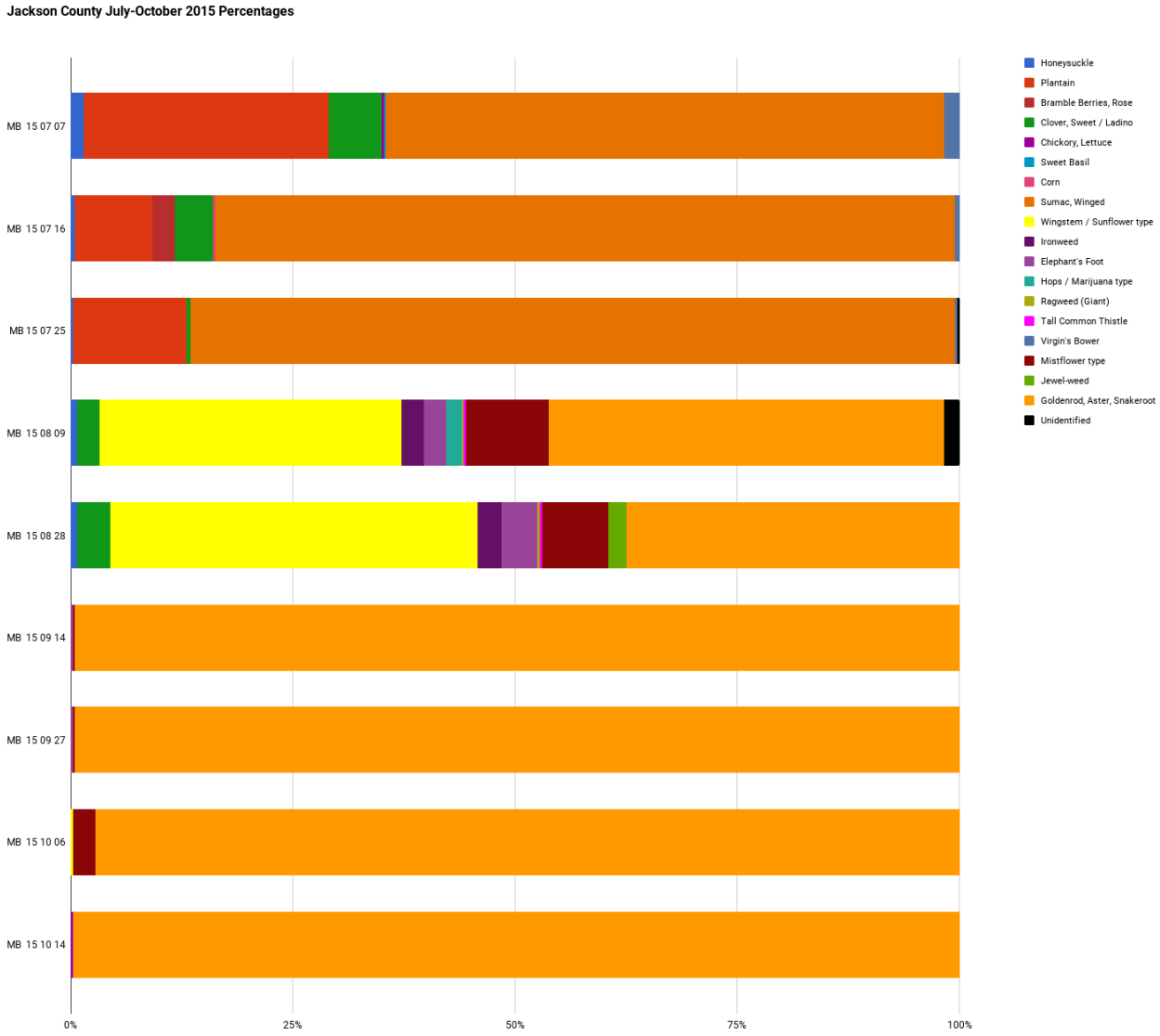
Prevalence Table: showing level of importance of Fall (July-October) Pollen Types in Your Neighborhood based on highest percentage found in samples analyzed:

Scientific Name	**Common Name**	**Highest % found**
Predominant Types		
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	100%
Rhus copallina	Sumac, Winged	86%
Secondary Types		
Verbesina / Helianthus	Wingstem / Sunflower type	41%
Plantago	Plantain	28%
Important Minor Types		
Conoclinium / Centaurea type	Mistflower / Knapweed type	9%
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	6%
Elephantopus	Elephant's Foot	4%
Rubus / Rosa	Bramble Berries, Rose	3%
Vernonia	Ironweed	3%
Minor Types		
Clematis virginiana	Virgin's Bower	2%
Humulus / Cannabis type	Hops / Marijuana type	2%
Impatiens capensis	Jewel-Weed	2%
Lonicera	Honeysuckle	1.5%
Cichorium / Lactuca type	Chickory, Lettuce	<1%
Ocimum basilicum	Sweet Basil	<1%
Zea mays	Corn	<1%
Ambrosia	Ragweed (Giant)	<1%
Cirsium altissimum	Tall Common Thistle	<1%

Table of Pollen Counts

Jackson County	2015	July						August				September/October							
Scientific Name	Common Name	MB 15 07 07		MB 15 07 16		MB 15 07 25		MB 15 08 09		MB 15 08 28		MB 15 09 14		MB 15 09 27		MB 15 10 06		MB 15 10 14	
Lonicera	Honeysuckle	6	2%	2	1%	1	0%	3	1%	3	1%	0	0%	0	0%	0	0%	0	0%
Plantago	Plantain	110	28%	35	9%	51	13%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Rubus / Rosa	Bramble Berries, Rose	0	0%	10	3%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Melilotus & T. repense	Clover, Sweet & Ladino	24	6%	17	4%	2	1%	10	3%	15	4%	0	0%	0	0%	0	0%	0	0%
Cichorium / Lactuca type	Chickory, Lettuce	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%
Ocimum basilicum	Sweet Basil	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Zea mays	Corn	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Rhus copallina	Sumac, Winged	251	63%	333	83%	344	86%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Verbesina / Helianthus	Wingstem / Sunflower type	0	0%	0	0%	0	0%	136	34%	165	41%	0	0%	0	0%	1	0%	0	0%
Vernonia	Ironweed	0	0%	0	0%	0	0%	10	3%	11	3%	0	0%	0	0%	0	0%	0	0%
Elephantopus	Elephant's Foot	0	0%	0	0%	0	0%	10	3%	16	4%	1	0%	1	0%	0	0%	0	0%
Humulus / Cannabis type	Hops / Marijuana type	0	0%	0	0%	0	0%	7	2%	0	0%	0	0%	0	0%	0	0%	0	0%
Ambrosia	Ragweed (Giant)	0	0%	0	0%	0	0%	1	0%	1	0%	0	0%	0	0%	0	0%	0	0%
Cirsium altissimum	Tall Common Thistle	0	0%	0	0%	0	0%	1	0%	1	0%	0	0%	0	0%	0	0%	0	0%
Clematis virginiana	Virgin's Bower	7	2%	2	1%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Conoclinium /Centaurea	Mistflower / Knapweed	0	0%	0	0%	0	0%	37	9%	30	8%	1	0%	1	0%	10	3%	0	0%
Impatiens capensis	Jewel-weed	0	0%	0	0%	0	0%	0	0%	8	2%	0	0%	0	0%	0	0%	0	0%
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	0	0%	0	0%	0	0%	178	45%	150	38%	398	100%	398	100%	389	97%	399	100%
	Unidentified	0	0%	0	0%	1	0%	7	2%	0	0%	0	0%	0	0%	0	0%	0	0%
		400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%

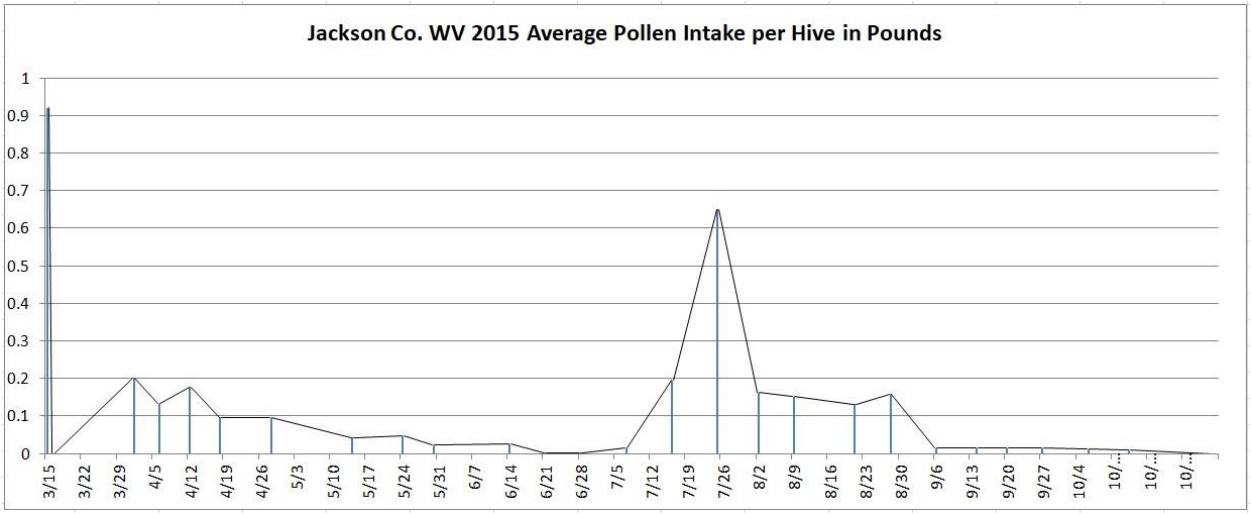
The information in the chart above is presented visually in the graph below. Each sample is listed on the vertical axis on the left. The percentages of each pollen type are listed along the horizontal axis in each bar. A separate color indentifies each pollen type. The graph shows the emergence and fading 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 MB 15-07-25, may represent a large amount of pollen while another bar such as MB 15-09-06 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 the first part of July, it was a relief to see pollen collection pick up again in the 2nd half of July and August. 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. Your location is an example of the drastic increase in pollen income sometimes seen that corresponds to the onset of Shining Sumac bloom *(Rhus copallina)*. The analysis showed that Sumac (probably Shining Sumac, also known as Winged Sumac) was indeed responsible for this drastic increase. I did not find fungus spores in the samples, which bees may forage in a pollen dearth.

Honey bees foraging on brown rot spores from peaches.

When pollen is scarce, bees will find whatever is available, including rust and fungus spores. The spike in pollen collection in July indicates a significant relief from the low levels in June and early July. I am not certain as to the protein content of Shining Sumac, but seeing what a significant part it played in the nutritional intake of the bees, it would be worth knowing. It seems logical that a strong pollen flow such as this would help the bees recover from the low amount of pollen gathered earlier, plus go a long way toward establishing a healthy protein reserve in the colony prior to raising the overwintering population. This reserve would exist both in the fat bodies of the individual bees, and as stored bee bread for access in future months. Traditionally, fall has been the chosen season to collect pollen from bees for human consumption or for sale. In a very good fall flow, such as that provided by shining sumac, it appears the bees might be able to share some of the bounty. At the same time however, this strong flow may not be dependable from year to year. Some beekeepers have noticed lower survivability in colonies from which pollen was harvested in the fall. In some locations, 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

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