WV Pollen Project 2017

Bee Pollen Collaborator Report – July through October samples

Steve Hamrick,

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, 22, and 30, August 17 and 31, September 15 and 30, and October 6, and 21 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:

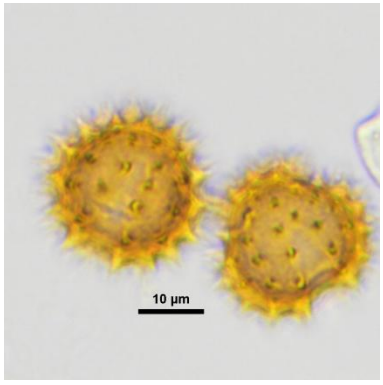
- Class A = >45%, called predominant pollen types
- Class B = 16-45%, called secondary pollen types
- Class C = 3-15%, called important minor pollen types
- Class D = <3%, called minor pollen types

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

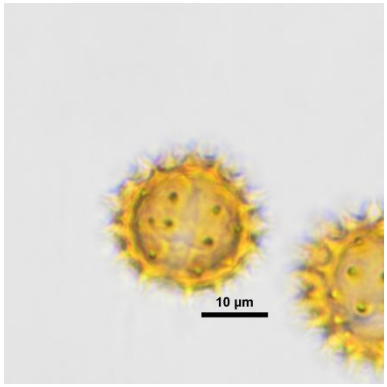
genus level without extensive reference collections and studies at levels of higher resolution scanning electron microscopy (SEM)."

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.

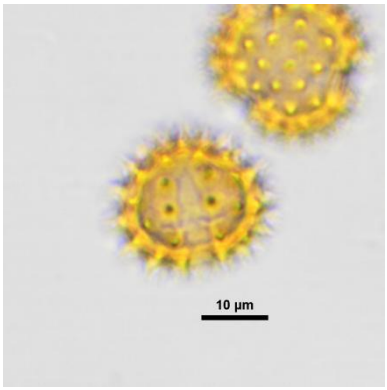
Below are some images of the pollen grains of Snakeroot, Goldenrod, and Aster showing their various features at a couple different angles.



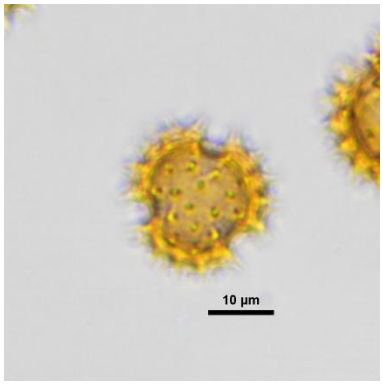
White Snakeroot (pore/furrow)



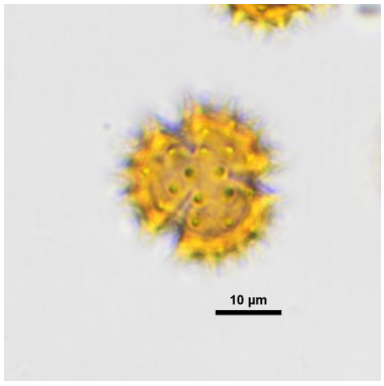
Tall Goldenrod (pore/furrow)



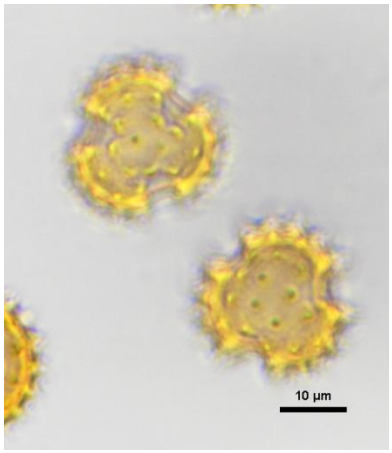
Late Aster (pore/furrow)



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 (SH 15-07-07)

You noted that plants in bloom when your July 7 sample was collected included White Clover, Sourwood, and possibly Sumac. I found the sample to contain 64.5% clover ("sweet clover" phenotype, which includes White Ladino Clover), 30% Virginia Creeper type, 5.25% other clover (red clover phenotype), and 0.25% unidentified.

Virginia Creeper pollen has been a significant pollen source in many summer samples. This would be a type worth knowing more about in terms of its protein and nutrient content.

The unidentified pollen grain in your sample was a tricolporate grain about 30 um in diameter with a thin exine and fine but very distinct reticulate grain on the surface which was much finer at the edges of the furrows. It held some resemblance to Sagebrush and Columbine pollen grains, but still different.

Clover pollen is known to contain levels of protein sufficient for colony growth.

July 22 (SH 15-07-22)

You noted that plants in bloom when you July 22 sample was collected included Jewel Weed. I found the sample to contain 82% corn, 11% clover (sweet / ladino phenotype), 2.5% plantain, 1.5% sumac, 1% unidentified, and 0.5% each of thistle, Virginia creeper, red clover, and cornflower.

The pollen grains of Sweet Corn and other types of corn are not distinguishable under the microscope. It is commonly said that bees much prefer sweet corn pollen over dent corn. One source says corn pollen is only 15% protein, while another puts it at 24%. The clover and other pollen types in your sample help balance the protein and other nutrients.

July 30 (SH 15-07-30)

You noted that plants in bloom when your July 30 sample was collected included Winged Sumac and Wingstem starting. I found the sample to contain 77% sumac, 13% clover (sweet / ladino phenotype), 5% Ironweed, 4% tanoak, 1% pokeweed, and traces of plantain and red clover.

It is common to find large quantities of Winged Sumac pollen at this time of year. There is usually little else blooming. However, the total pollen intake for this sample was very small, which could indicate that the plant is scarce in your area.

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

August 17 (SH 15-08-17)

You noted that plants in bloom when your August 17 sample was collected included Wingstem, Japanese Knotweed, Joe-Pye-Weed, and Ironweed. I found the sample to contain 58.75% clover (sweet / ladino phenotype), 12.25% plantain, 10.75% wingstem, 7.75% corn, 6.75% blackberry type, 1.5% elephant's foot, 0.75% goldenrod / snakeroot type, 0.5% lettuce type, 0.5% sumac, 0.25% red clover, and 0.25% Ironweed.

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.

The Blackberry-like pollen grains were puzzling. They could be a purple-flowering raspberry *(Rubus odoratus)* native to West Virginia that blooms in July and August.

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.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting and found a small amount of Thistle, Virgin's Bower, and Wild Carrot pollen, but not enough to amount to any appreciable percentage of the sample.

With clover as a fairly good source of protein and other types for variety and balance, the bees diet looks okay at this point in time. 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.

August 31 (SH 15-08-31)

You noted that plants in bloom when your August 31 sample was collected included Wingstem, Ironweed, Clematis, and Evening Primrose, with Japanese Knotweed ending. I found the sample to contain 36.5% wingstem, 35.75% goldenrod / snakeroot type, 7.25% dock weed, 5.75% plantain, 5.25% ragweed, 3.75% clover (sweet / ladino phenotype), 2.75% jewel weed, 1.5% elephant's foot, 0.5% red clover, 0.25% corn, 0.25% grass, 0.5% from a couple unidentified (or possibly deformed) types.

Of the 5.75% Plantain pollen, 5.5% was from English plantain and the other 0.25% from Broadleaf plantain.

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

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 a type of smartweed, but not enough to amount to any appreciable percentage of the sample.

Nearly 80% of the sample came from species within the Asteraceae family (Wingstem, Goldenrod/Eupatorium, Ragweed, and Elephant's Foot). 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. It is hard to tell what high quality pollen could remain from earlier in the year, stored in the hive as bee bread.

September 15 (SH 15-09-15)

You noted that plants in bloom when your September 15 sample was collected included two types of goldenrod, and jewel weed, while Wingstem and ironweed were ending. I found the sample to contain 74.25% goldenrod / snakeroot type, 25% Wingstem type, and 0.75% corn pollen.

Pollen samples dominated by the goldenrod type typically coincide with the onset of fall-blooming goldenrod, known as Canada goldenrod or Tall Goldenrod. This 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

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 30 (SH 15-09-30)

You noted that plants in bloom when your September 30 sample was collected included White Snakeroot and Aster, with Goldenrod about 90% finished. I found the sample to contain 90.75% goldenrod / aster / snakeroot, 7.5% Wingstem, 1% clover (sweet / ladino phenotype), 0.5% elephant's foot, and 0.25% lettuce / chicory.

The total volume of pollen collected on this date was very low, indicating that after the end of goldenrod bloom the bees had very few pollen resources.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting. I found a red clover pollen grain, a honeysuckle pollen grain, and a few *Dianthus* type grains which could have been from Sweet William, Deptford Pink, or Carnation flowers.

October 6 (SH 15-10-06)

You noted that Asters were in bloom when your October 6 sample was collected, with Goldenrod almost done. I found the sample to contain 90.5% Goldenrod / Snakeroot / Aster pollen, 5.5% wingstem / sunflower, 2.5% Mistflower / Knapweed, 1% Sweet William type, 0.25% Cocklebur, and 0.5% Lettuce / Chicory.

After counting the random 400 grains, I scrolled around the slide to see if there was anything else interesting. I found a clover, a honeysuckle, an elephant's foot, an oxalis, and one that looked like honeysuckle except without any spines and a much finer (almost smooth) granular surface pattern.

It is interesting to see an increase in the average amount of pollen collected per hive on this date compared to September 30. It is probably safe to assume that most if not all of the "goldenrod / snakeroot / aster" type pollen in this sample is from Asters.

October 21 (SH 15-10-21)

You noted that you were uncertain as to what plants were in bloom when your October 21 sample was collected. I found the sample to contain 93.5% aster / goldenrod / snakeroot type pollen, 4.5% Chrysanthemum type, 1.75% sweet William type, and 0.25% lettuce / chicory.

Once again asters most likely account for the 93.5%. The appearance of Chrysanthemum pollen is interesting, possibly from an ornamental planting.

At this time of year the bees' winter preparations should be complete for the most part and what little pollen they are able to find is incidental.

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

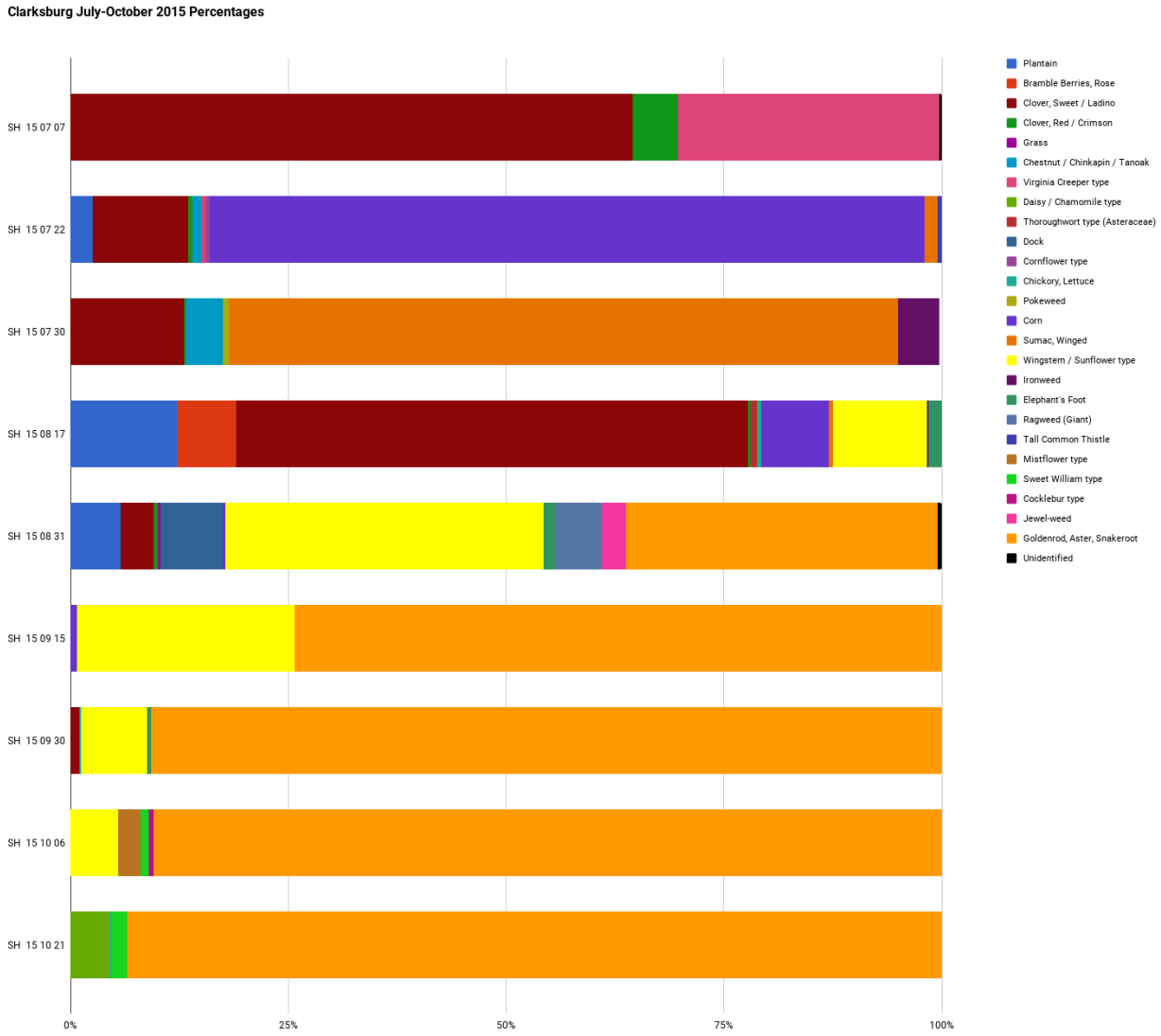
Scientific Name	Common Name	Highest % found
Predominant Types (>45%)		
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	94%
Zea mays	Corn	82%
Rhus copallina	Winged (Shining) Sumac	77%
Melilotus & Trifolium repens	Sweet & Ladino Clover	65%
Secondary Types (16-45%)		
Verbesina / Helianthus	Wingstem / Sunflower type	37%
Parthenocissus	Virginia Creeper	30%
Important Minor Types (3-15%)		
Plantago	Plantain	12%
Rubus / Rosa	Brambles / Rose	7%
Rumex	Dock	7%
Trifolium	Red Clover	5%
Chrysanthemum / Matricaria	Daisy / Chamomile type	5%
Vernonia	Ironweed	5%
Ambrosia	Ragweed (Giant)	5%
Castanea / Notholithocarpus	Chestnut / Tanoak	4%
Conoclinium / Centaurea type	Mistflower / Knapweed type	3%
Impatiens capensis	Jewelweed (Touch-Me-Not)	3%
Minor Types (<3%)		
Elephantopus	Elephant's Foot	2%
Dianthus	Sweet William Type	2%
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	<1%
Phytolacca	Pokeweed	<1%
Cichorium / Lactuca type	Chicory / Lettuce	<1%
Xanthium type	Cocklebur type	<1%
Poaceae	Grass	<1%
Centaurea cyanus type	Cornflower type	<1%
Cirsium altissimum	Tall Common Thistle	<1%

Table of Pollen Counts

Clarksburg	2015	July						August				September/October							
Scientific Name	Common Name	SH 15 07 07		SH 15 07 22		SH 15 07 30		SH 15 08 17		SH 15 08 31		SH 15 09 15		SH 15 09 30		SH 15 10 06		SH 15 10 21	
Plantago	Plantain	0	0%	5	3%	1	0%	49	12%	23	6%	0	0%	0	0%	0	0%	0	0%
Rubus / Rosa	Bramble Berries, Rose	0	0%	0	0%	0	0%	27	7%	0	0%	0	0%	0	0%	0	0%	0	0%
Melilotus & T. repens	Clover, Sweet & Ladino	258	65%	22	11%	52	13%	235	59%	15	4%	0	0%	4	1%	0	0%	0	0%
Trifolium	Clover, Red / Crimson	21	5%	1	1%	1	0%	1	0%	2	1%	0	0%	0	0%	0	0%	0	0%
Poaceae	Grass	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%
Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	0	0%	2	1%	17	4%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Parthenocussus	Virginia Creeper type	120	30%	1	1%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Chrysanthemum / Matricaria	Daisy / Chamomile type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	18	5%
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	0	0%	0	0%	0	0%	3	1%	0	0%	0	0%	0	0%	0	0%	0	0%
Rumex	Dock	0	0%	0	0%	0	0%	0	0%	29	7%	0	0%	0	0%	0	0%	0	0%
Centaurea cyanus type	Cornflower type	0	0%	1	1%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Cichorium / Lactuca type	Chickory, Lettuce	0	0%	0	0%	0	0%	2	1%	0	0%	0	0%	1	0%	0	0%	1	0%
Phytolacca	Pokeweed	0	0%	0	0%	3	1%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Zea mays	Corn	0	0%	164	82%	0	0%	31	8%	1	0%	3	1%	0	0%	0	0%	0	0%
Rhus copallina	Sumac, Winged	0	0%	3	2%	307	77%	2	1%	0	0%	0	0%	0	0%	0	0%	0	0%
Verbesina / Helianthus	Wingstem / Sunflower type	0	0%	0	0%	0	0%	43	11%	146	37%	100	25%	30	8%	22	6%	0	0%
Vernonia	Ironweed	0	0%	0	0%	19	5%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Elephantopus	Elephant's Foot	0	0%	0	0%	0	0%	6	2%	6	2%	0	0%	2	1%	0	0%	0	0%
Ambrosia	Ragweed (Giant)	0	0%	0	0%	0	0%	0	0%	21	5%	0	0%	0	0%	0	0%	0	0%
Cirsium altissimum	Tall Common Thistle	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Conoclinium / Centaurea	Mistflower / Knapweed	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	10	3%	0	0%
Dianthus	Sweet William type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	4	1%	7	2%

Scientific Name	Common Name	July 7		July 22		July 30		Aug. 17		Aug. 31		Sept. 15		Sept. 30		Oct. 06		Oct. 21	
Xanthium type	Cocklebur type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	1%	0	0%
Impatiens capensis	Jewel-weed	0	0%	0	0%	0	0%	0	0%	11	3%	0	0%	0	0%	0	0%	0	0%
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	0	0%	0	0%	0	0%	0	0%	143	36%	297	74%	363	91%	362	91%	374	94%
	Unidentified	1	0%	0	0%	0	0%	0	0%	2	1%	0	0%	0	0%	0	0%	0	0%
		400	100%	200	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%

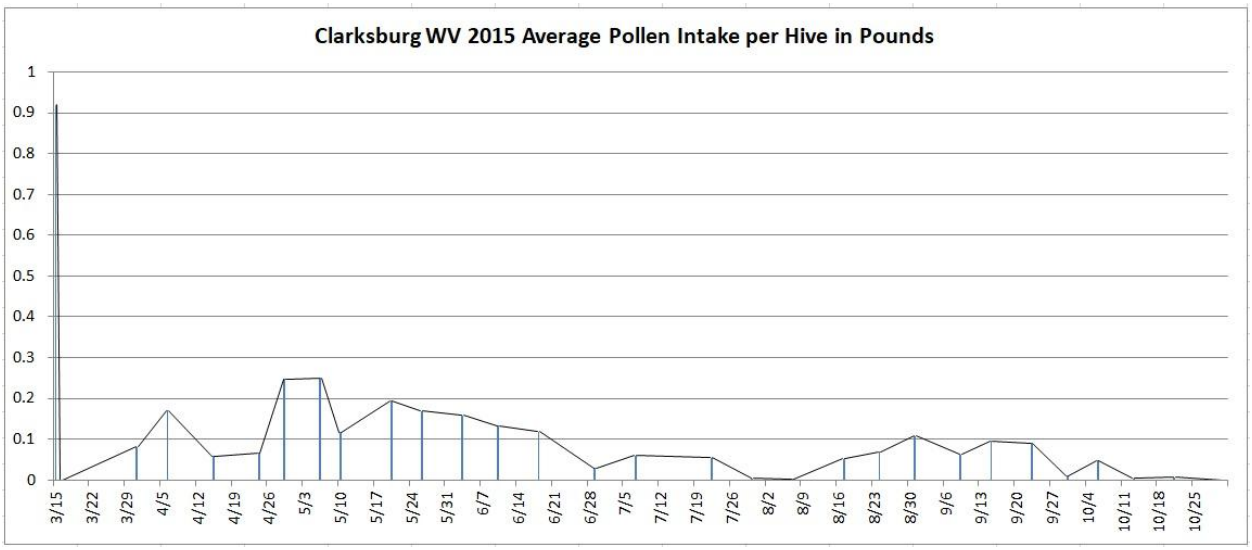
The information in the chart above is presented visually in the graph below. Each sample is listed on the vertical axis on the left. The percentages of each pollen type are listed along the horizontal axis in each bar. A separate color indentifies each pollen type. The graph shows the emergence and fading 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 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 and early August, it was a relief to see pollen collection pick up again in late 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)*. While most of your July 30 sample did consist of Sumac, it is interesting that the total pollen income on that date was very small. 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 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.