



Defining Honey Bee Pollen Sources in Appalachia

July through October Final Outreach Report.

Northeast SARE Farmer Grant 2017

Michael Staddon, Project Leader

If any post-grant revisions are made to this document, the most updated version will be posted at <https://www.honeyglen.com/2018/12/23/defining-honey-bee-pollen-sources-in-appalachia-july-through-october/>

Summary

The purpose of the West Virginia Pollen Project was to begin gathering factual and comprehensive information on where honeybees obtain their nutrition (i.e. pollen) in West Virginia's natural ecology. Studies such as this are the first step in assessing the normal nutritional condition of honey bee colonies in our area so that producers, researchers, and authorities can understand which plants are actually most important to honey bees and can make informed management decisions.

On a regular basis through the 2015 active season, collaborating beekeepers trapped pollen pellets at hive entrances at five locations in West Virginia and sent pellet samples to the project leader. In 2015, selected samples were prepared for analysis by Professor Bryant at Texas A&M University, after which 48 samples from the months of March through June were analyzed, graphed, and reported by the project leader to show the percentage of each pollen type in the March through June samples. The same process was repeated for 62 July through October samples in 2017 and 2018 through a second grant, "Defining Honey Bee Pollen Sources in Appalachia July through October". The results of the July through October analysis are laid out below.

The data showed both similarities and significant differences between locations, as well as between different years in the same location. At times a single pollen type comprised 100% of a sample, while at other times no one type made up more than 30%. Sometimes we saw exactly what we expected, such as fall-blooming Goldenrod (*Solidago canadensis*) in September, and at other times we saw very unexpected results such as nearly 60% of a sample comprised of Chenopodium / Amaranth (probably Lambsquarters, *Chenopodium album*) in July. The main challenge to the project was the project leader finding time to complete the project while working two other jobs simultaneously.

Introduction

Having been involved in beekeeper education since 2007, the project leader became aware of misconceptions and many unanswered questions in the area of honey bee and plant relationships, especially in Appalachia's diverse natural ecosystem. As a small beekeeping operation that produced and sold both local honey and nucleus colonies it was felt that working toward resolving the mystery surrounding the nutritional state of honey bees in West Virginia would be well worthwhile. Furthermore, before investing resources to assess the protein or nutrient content of the pollen types most used by bees, we needed to establish which plants are in fact most used by bees. For instance, it would be counter-productive to find the nutrient content of Japanese Knotweed pollen only to discover later that it never comprises more than 2% of the honey bees' diet.

The samples for the study were collected from 5 locations in West Virginia; three in Harrison County, one in Jackson County, and one in Raleigh County at 3,000 feet elevation. We were thrilled that Professor Vaughn Bryant, Palynologist at Texas A&M University, agreed to fill the role of technical advisor.



Objectives / Performance Targets

In order to make the project useful, we needed to not only gather factual information on honey bee foraging, but also share it with beekeepers in an easy-to-understand format. Colored bar-graph charts showing the percentage of each pollen type gathered on each given date at each location were our primary end product. Our aim was to display the facts in such a way that individuals could see answers to the following types of questions:

- What pollen types are the bees actually collecting?
- What is the percentage of each type at each time period?
- When are the bees bringing in the highest and lowest quantities?
- How much does it change from one location to the next?
- How much does it change from one year to the next?
- Which wild plant species or types are most valuable to honey bee nutrition and health?

Materials and Methods

Pollen Collection:

Samples were collected in 2015. Supplies used included pollen traps, plastic vials, and weighing scales. The pollen traps were attached to the beehives and pollen collection began during the Maple flows in March and continue throughout the year.

Collaborators were recruited in the fall just prior to the grant writing process and were made aware of the plans and procedures that would be followed pending approval. It was required that the collaborators' colonies be located close to their residence so that the traps could be worked easily on a daily basis.



Pollen was to be collected from at least three colonies on each collection date as much as practicable. This is because the project leader had occasionally seen different hives, on the same day and at the same location, collect significantly different percentages of the various pollen types (judging by the color of the pellets). The image shows the pollen from three traps collected on the same day at the same location with different color combinations. By mixing the harvest from three colonies collected on the same day, we would obtain a good overall picture of what bees were collecting on that date.

Each collaborator was provided with four pollen traps. This way, even if one colony failed (i.e. colonies could swarm or collapse), and its trap needed to be moved to a different colony, a minimum of three traps could still be in use. When moving a trap to a new colony it may take a week's time after moving a trap before any pollen could be collected from the new colony.

Collaborators were also required to have a spring minimum of six healthy colonies in the home apiary, and preferably more. This would ensure that backup colonies would be available if needed.

Some of the collaborators volunteered to take two samples per month, others volunteered to take one sample each week, or about four samples per month. The collaborators were free to choose their own collection dates according to the weather conditions at their location, as long as they fulfilled the number of samples promised. Collection dates at regular intervals through the year was the goal. All the collaborators were provided with an instruction sheet detailing the standard pollen sampling process.

The collaborators also needed some freezer space for keeping samples until they were sent to the project leader, and access to a reliable weather forecast to aid in selecting the best dates for pollen collection.

The type of pollen trap used was the plastic front porch pollen trap such as sold by Betterbee and Brushy Mountain. The project leader had tested two other pollen trap styles – the Sundance bottom board trap and the wooden front porch style with the “asterisk strip” – and found the plastic front porch type to be the only one suited to our purpose. The problem with the wooden front porch traps using the “asterisk” holes in the trapping strip was that, when used in a manner as for our sampling purposes, they consistently failed to trap enough pollen to get a reasonable picture of the volume being collected. This was mainly due to the bees refusing to pass through the strip and ending up bearding outside the trap much more stubbornly than was the case with the plastic front porch trap. The problem with the traditional Sundance type bottom board traps was that they were designed for continuous trapping which we felt would alter the bees' foraging habits, perhaps significantly, especially in terms of the quantity of pollen collected. The path the bees must travel during trapping is altered to such an extent that when switched to trapping mode for only one day per week, the bees are not able to learn the new route well enough to be effective.

A limitation that would be faced with any pollen trap on the market would be that some pollen will make it through the trap without being removed from the bees' legs. This would be especially true when smaller loads were being gathered. Pollen from any time period or pollen type for which the bees only collected small loads may go entirely undetected by the project. In other words, analysis cannot be truly conducted on 100% of the pollen collected by bees.

Collaborators attached the traps to the front of their hives and allowed the bees to adjust to the trap for a few days to a week without collecting pollen. The traps' drone escapes were plugged or covered during that time to ensure that the bees oriented to the main entrance only. It was recommended that the collaborator custom cut small pieces of wood for keeping the trap-gates open or closed at the appropriate times. After orientation was complete or at the first collection date, the drone escapes were opened for their intended use.



The biggest difficulty in bee orientation to the trap was confusing the top of the trap with the landing board. Temporarily constructing a false front helped greatly with this issue.

To collect pollen, the collaborators first chose the best day of each week, which meant temperatures as close as possible to the ideal range of 70 to 90 degrees Fahrenheit (21 to 32 degrees Celsius), and no chance of rain.

Traps were closed to begin trapping pollen in the morning and opened to cease trapping a few hours before dark. The pollen collection drawers were wiped clean prior to each trapping.

If the bees refused to go through the trap-gate when it was closed for trapping, they would end up bearding on the outside of the trap. This situation improved with continued trapping. It was sometimes helpful to open the trap-gates to allow bees to re-enter the hive ½-hour before collecting the pollen. This gave the bees time to get back inside, making harvest easier. Sometimes on hot summer evenings the bees were not interested in entering the hive, and had to be brushed off the trap drawer to harvest the pollen.

Pollen traps were left open (non-collection mode) between sampling dates. This helped to minimize any altered pollen collection behavior that might be caused by continuous trapping (i.e. bees attempting to compensate for the trapping by collecting far more pollen).

The pollen from all the traps in one day's collection was brought indoors and thoroughly mixed in such a way that the pellets were not damaged. It was extremely important for the accuracy of the study that the pollen from all the traps be mixed very thoroughly. It was also very important to harvest the pollen and bring it indoors on the same evening that it was trapped (not allow it to remain in the trap drawer on the hive overnight) to prevent the nighttime dew and dampness from spoiling the pollen, or at least making the pellets sticky and difficult to mix.

The collaborators were provided with "pollen sample record sheets" to print and use for recording important information with each sample. The total pollen harvest was weighed, and the weight divided by the number of traps used to find the average weight of pollen harvested per hive. Both numbers were entered into a record sheet along with the collaborator's name, the date, the number of traps used, information on the weather, the times that the traps were closed and opened, and notes on any plants in bloom at the time of collection that the collaborator was aware of.

Two vials were filled with the pollen and labeled with the date and enclosed along with the record sheet in a sealable bag. If a total of less than two ounces of pollen were collected on a given date, the collaborator would include whatever was harvested. Even if only a single pellet was collected in all the traps together, it is possible to conduct analysis and find its source. Whatever the bees collected above the two ounces, the collaborator was free to use for his own purposes.

All containers used to mix and weigh pollen were washed between collections so as to avoid cross contamination between samples.



One sample consisted of two 1-oz vials of pellets, taken from the mixed pollen of 3 or more traps in one apiary, trapped throughout one daylight period, labeled with the date, and contained in a sealable bag with a completed Pollen Sample Record Sheet.

If a colony from which pollen was being trapped began to bring in less pollen than would have been expected of a healthy colony, the collaborators were encouraged to transition the trap to another colony. No data on colony health were recorded for the project.

At the start of each month the pollen samples from the previous month were mailed or delivered to the project leader. Until then the pollen samples were kept frozen to maintain freshness.

The pollen collection process went mostly as planned. Rarely a sample was taken from less than three traps. Three of the collaborators were from Harrison county at roughly the same latitude (39) and between 1,000 and 1,200 ft. elevation. The fourth collaborator was at Ripley in Jackson County at 750 ft. elevation and the fifth at Cool Ridge in Raleigh County at about 3,000 ft. elevation.

Over the course of the project a total of 121 samples were collected. The project leader also submitted 55 samples taken from two previous years in the same location. This would provide information that would not only allow us to compare pollen foraging between different locations, but also between different years in the same location.

Preparation of Samples:

62 of the submitted samples were chosen for analysis from the months of July through October. The previous Grant, titled the “West Virginia Pollen Project” has already reported on analysis of the March through June samples. So as to form an optimal picture of pollen gathering at regularly spaced dates through the season, usually two samples per month were chosen from each collaborator at evenly spaced dates for the months of July through October.

Because of honey bees’ plant species fidelity while foraging, each pellet typically represents only one plant type. If only 1% of the foragers from the hive were foraging on a particular plant type, approximately 1% of the pellets in the trap drawer would be from that pollen source. If only 10 pellets are taken from the sample and prepared for analysis, the 1% type would likely be left out and never detected. But if one pellet from that type was included in the 10, it would show up as 10% of the sample in the analysis – ten times its actual constituency! **It is impossible for the analysis to be more accurate than the initial number of pellets included.** The project leader has seen a pollen pellet of a distinct color alone among a thousand pellets – but did not feel a need for this level of accuracy. **We decided to have a subsample of at least 200 pellets, or 2 grams, included in each sample’s preparation for analysis,** which we felt would give us a meaningful level of accuracy for our purposes. A single pellet among the 200 would represent 0.5% of the sample. Anything less than 3% of a sample is considered a “minor” pollen type.

It is also possible for pollen grains to blow onto a bee or flower and inadvertently become packed into a bee's pollen pellet. Pine pollen is an example of a very low-protein-content pollen that bees usually ignore, but which blows through the air abundantly while in bloom.

The project leader measured out **subsamples of 5 grams of pellets** from each of the 62 samples and sent them to Texas for preparation. The 5 grams would allow plenty of pollen to prepare the sample a second time if needed. The project leader froze the remaining pellets from the 2-oz samples submitted by the collaborators for future reference.

The pollen preparation procedure was carried out at Texas A&M University by Professor Vaughn Bryant. The treatment removes lipids, waxes, and cytoplasm from the pollen grain's exterior to allow for accurate identification under the microscope. Think of a pollen grain like a clingstone peach pit; you can tell its general shape without removing all the flesh, but after all the flesh is removed, the details of the grooves and pits in the shell are much more clearly seen.

To briefly summarize Professor Bryant's preparation process, 2 grams were taken from the 5-gram subsample (>200-pellets) and thoroughly dissolved and mixed in glacial acetic acid so that a much smaller extracted amount (4-5 ml) could be taken that would still represent the same pollen type ratios. This sample was then treated with acetolysis (sulfuric acid and acetic anhydride) to remove the lipids, waxes and cytoplasm. Most of the samples were then stained to provide contrast for microscopic analysis and photography. The pollen was rinsed in ETOH and then mixed with glycerin and sealed in a vial for shipping back to the project leader. At each step of the preparation process, the samples were centrifuged at 3,500 rpm for 3 minutes.

Pollen Sample Analysis



Upon receipt of the prepared pollen, each vial was stirred and a toothpick inserted and allowed to stand for about 24 hours to allow any extra ETOH to evaporate.

To mount the pollen to the slides, the vial was stirred and shaken for 1 minute and a small amount of the pollen solution was placed on a slide, diluted with additional glycerin when needed, and covered with a coverslip. Just enough solution was needed to spread out under

most of the coverslip, ideally covering about 90 or 95% of the area. The edges of the coverslip were sealed with clear nail polish. If there was too much solution under the slide there were issues with poor sealing and pollen grains moving. If not enough solution was used, the solution gravitated to the outside edges and left the pollen grains in pockets. When not in use, the slides were stored in small plastic "slide

mailers" which kept dust out and allowed them to be stored horizontally to prevent leakage. Extra pollen-glycerin solution was kept for future reference.



WVU provided a loan of a VWR light microscope for use in the pollen analysis work with 4x, 10x, 40x, and 100x oil objectives. There were no 60x objectives available so the pollen identification and counting was conducted with the more common 100x oil immersion and 40x objective lenses. These provided 1,000x and 400x magnifications.

The 10x and 4x lenses were helpful for finding locations on a slide more quickly when needed.

To conduct the analysis, 400 pollen grains were counted and identified in each sample to the family, genus, or in some cases species level to establish a valid relative abundance for each type. Typically, the count started near the bottom right corner of the slide and progressed toward the center of the slide until 400 pollen grains had been counted. Other areas of the slide were also scanned to see if there were any noteworthy pollen grains of low abundance. References that proved very helpful for pollen grain identification were Pollen and Spores by Ronald O. Kapp, 2nd edition, An Atlas of Airborne Pollen Grains and Common Fungus Spores of Canada (Canada Department of Agriculture), the PalDat web site, and a large number of digital images supplied by Professor Bryant of Texas A&M University. Some helpful information was also found in Lindtner's Garden Plants for Honey Bees.

For some pollen types for which references did not exist or were insufficient, the project leader was able to collect pollen pellets from honey bees working that species and have the pollen treated so that he could view it and be more confident of the identifications.

Professor Bryant 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 in 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)."

When analysis of the July through October samples was complete, the project leader compiled a report for each collaborator detailing the analysis procedure, comments on the findings, and charts showing the relative abundance of each type found in their specific samples. Some photographs of the pollen found were also included.

The charts and graphs were developed in Google Sheets. The Chrome browser allowed the bar graphs to be easily saved as images for inclusion in the reports.

Having previously completed analysis on the March through June samples the 2015 Pollen Project grant, the project leader had a better feeling for the volume of time required for the work. Time demands during the busy April, May, and June beekeeping season in 2018 nearly eliminated work during that time. In June 2018 the project leader also took a full time job at a beekeeping operation that required some commuting, and was able to do work on the project while car-pooling for the commute. The project leader also became engaged to be married in August and needed to spend significant amounts of time communicating with his fiancée, who also helped in editing the final report.

Outcomes and Impacts

From the data collected we were able to graph an accurate, comprehensive, verifiable picture of pollen intake for each location where pollen was collected for the months July through the end of the pollen collecting season in October. Tables and graphs were created to answer the questions listed in the Project Summary. The best understanding of the results, and answers to individual questions, are best obtained by viewing the tables and graphs.

The **Pollen Percentage Tables and Bar Graphs** show the percentages of each pollen type in each sample. This data helps us determine which species are contributing most to the bees' nutritional intake at various times through the year. The bar graphs often helped visualize the changes that took place as different species came into bloom and faded away over time. Graphs from different locations, and the graphs from different years at the Salem location can be easily compared.

The **Average Pollen Intake Graphs** show the total amounts of pollen intake on a per hive basis at each collection point through the whole season so that beekeepers can see clearly when high or low amounts of pollen were being gathered by the bees.

The **Pollen Importance Tables** organize the pollen types found by level of importance based on both highest percentage found in any sample, and on highest amount in weight gathered per hive at any one time. One table is organized in descending order based on percentage, the other is organized in descending order based on amount gathered by weight. These tables show, based on our data, the highest degree that each taxa currently contributes to the bees' pollen intake, considering all samples analyzed from all locations.

The colored bar graphs and related comments were supplied to the collaborating beekeepers so that they could in turn share them with other beekeepers in their local areas, who share the same ecologies.

Analysis of all the samples was finished in time to deliver a summary presentation of the results at the at the West Virginia Beekeepers Association (WVBA) annual executive board meeting on November 3, 2018 in Flatwoods, West Virginia. The WVBA newsletter editor requested a written form of the presentation to be submitted for inclusion in the WVBA spring newsletter, which was done. The newsletter will be published and distributed in the early spring of 2019.

A final outreach report including the tables and bar graphs was compiled into a PDF document and posted on the Honey Glen Blog, www.honeyglen.com, as well as submitted to the West Virginia Beekeepers Association web master for posting on the WVBA website.

Beekeepers and interested individuals throughout West Virginia were notified of the completion of the project and where the final outreach report could be found.

Beekeepers in the surrounding states of Virginia, Maryland, Pennsylvania, Ohio, and Kentucky were also notified of the completion of the project.

An article was submitted to the American Bee Journal, expected to be published in March or April 2018.

Anyone interested in planting for pollinators, whether that be Beekeepers, Landowners, Land Reclamation Specialists, Bioengineers, Landscapers, Utility Companies, Wildlife Biologists, or others can add this information to existing knowledge. It may help with assessment of an area's pollinator forage to see what is already abundant and if it provides pollen for bees, find what gaps need to be filled, and know which species actually do or do not provide the pollen on which insect pollinators depend.

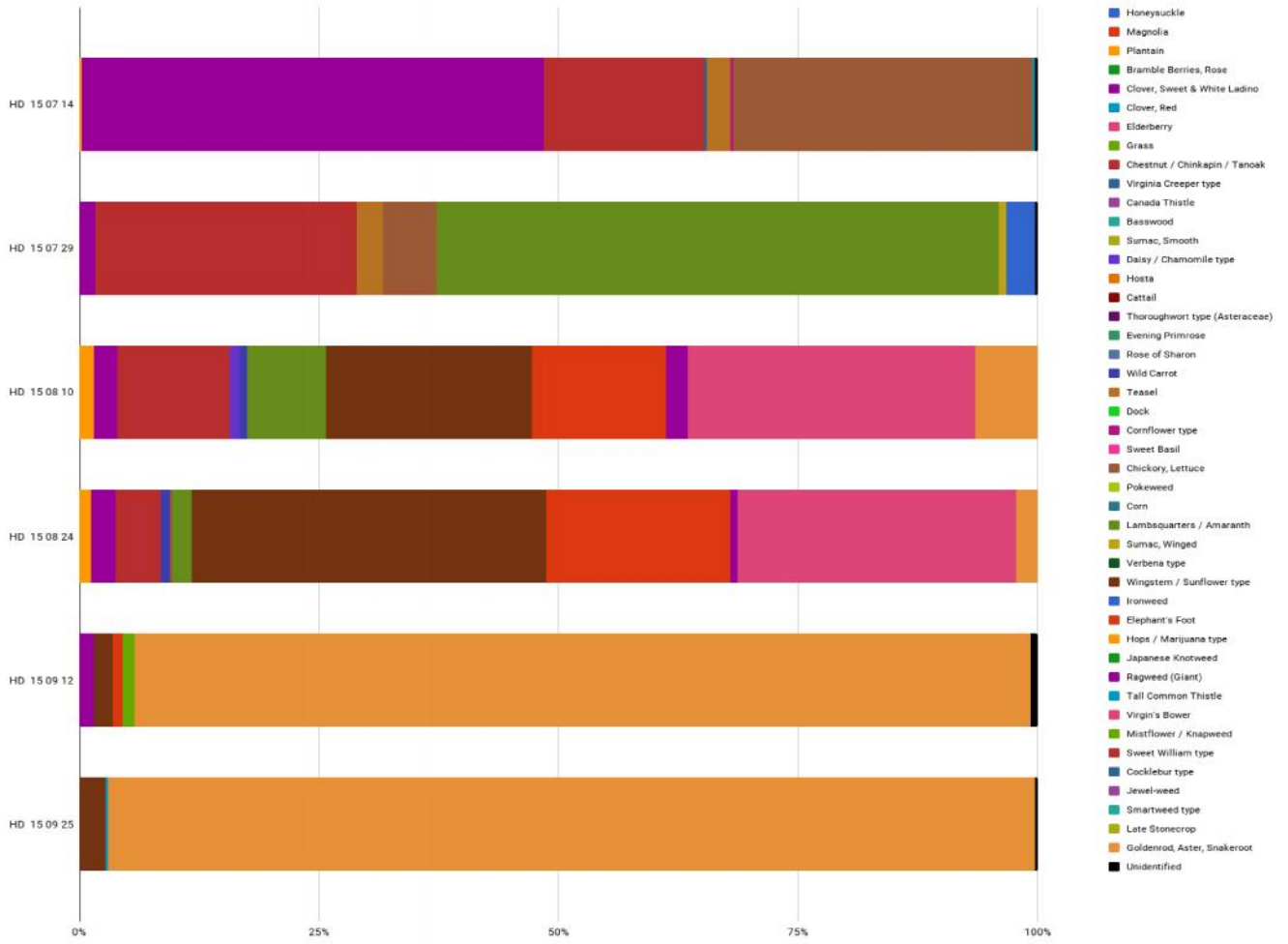
Anyone interested in the biology behind the dynamics of colony population and health in the mid-Appalachian region will benefit from a fact-based understanding of pollen income.

Pollen Percentage Tables and Bar Graphs: Percentages of each pollen type by date, July through October, rounded to nearest 1%. Plant list organized by approximate bloom time.

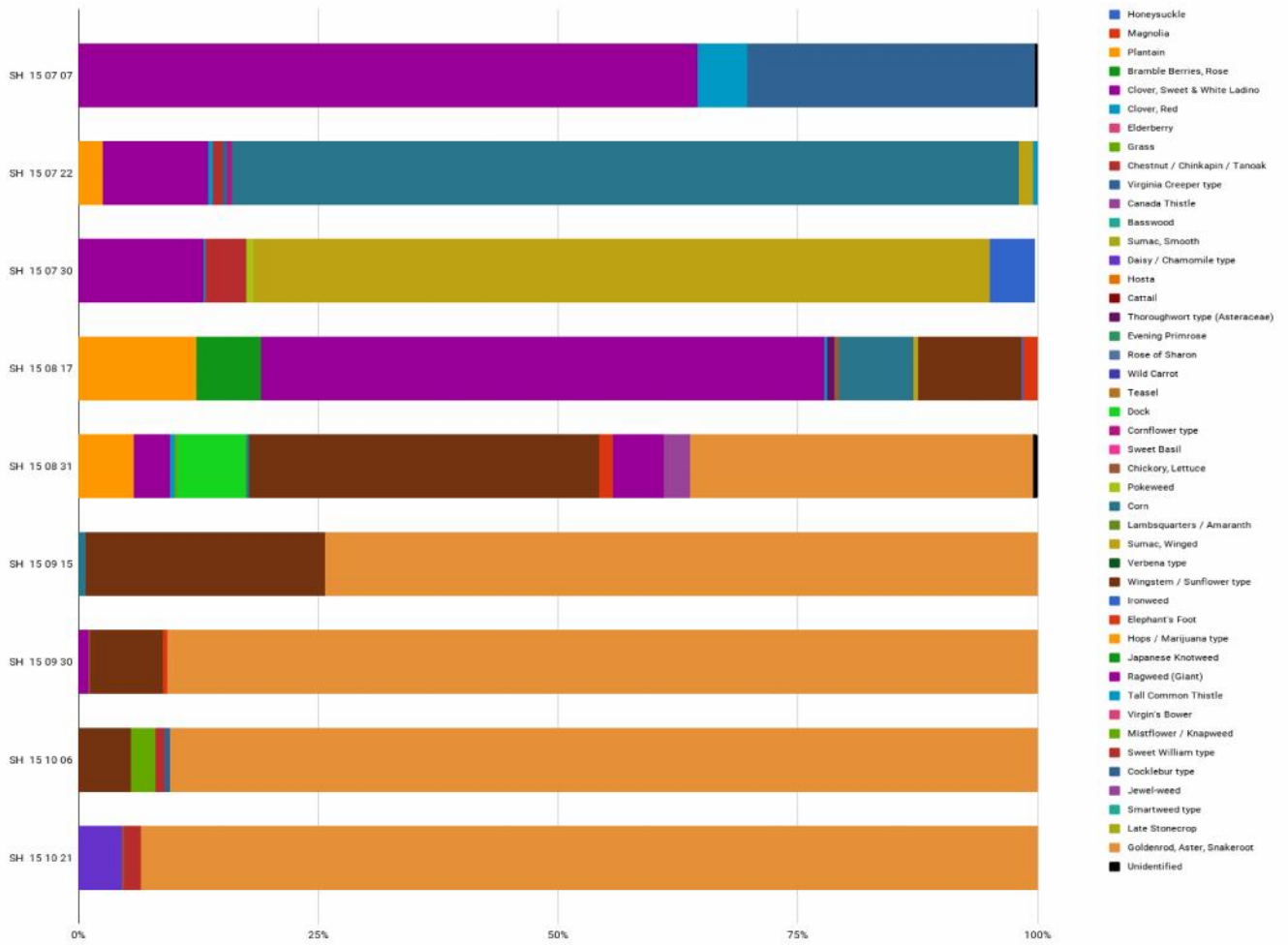
Quiet Dell WV, Harrison County. 1080 ft. elevation, Latitude 39.226.

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
Lonicera	Honeysuckle	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Magnolia	Magnolia	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Plantago	Plantain	1 0%	0 0%	6 2%	5 1%	0 0%	0 0%
Rubus / Rosa	Bramble Berries, Rose	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	193 48%	7 2%	10 3%	10 3%	6 2%	0 0%
Trifolium pratense	Clover, Red	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Sambucus	Elderberry	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Poaceae	Grass	0 0%	0 0%	0 0%	0 0%	0 0%	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%
Cirsium arvense	Canada Thistle	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Tilia	Basswood	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Rhus glabra	Sumac, Smooth	0 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%
Hosta	Hosta	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Typha	Cattail	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Oenothera, likely speciosa	Evening Primrose	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Hibiscus syriacus	Rose of Sharon	0 0%	0 0%	0 0%	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%
Rumex	Dock	0 0%	0 0%	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%

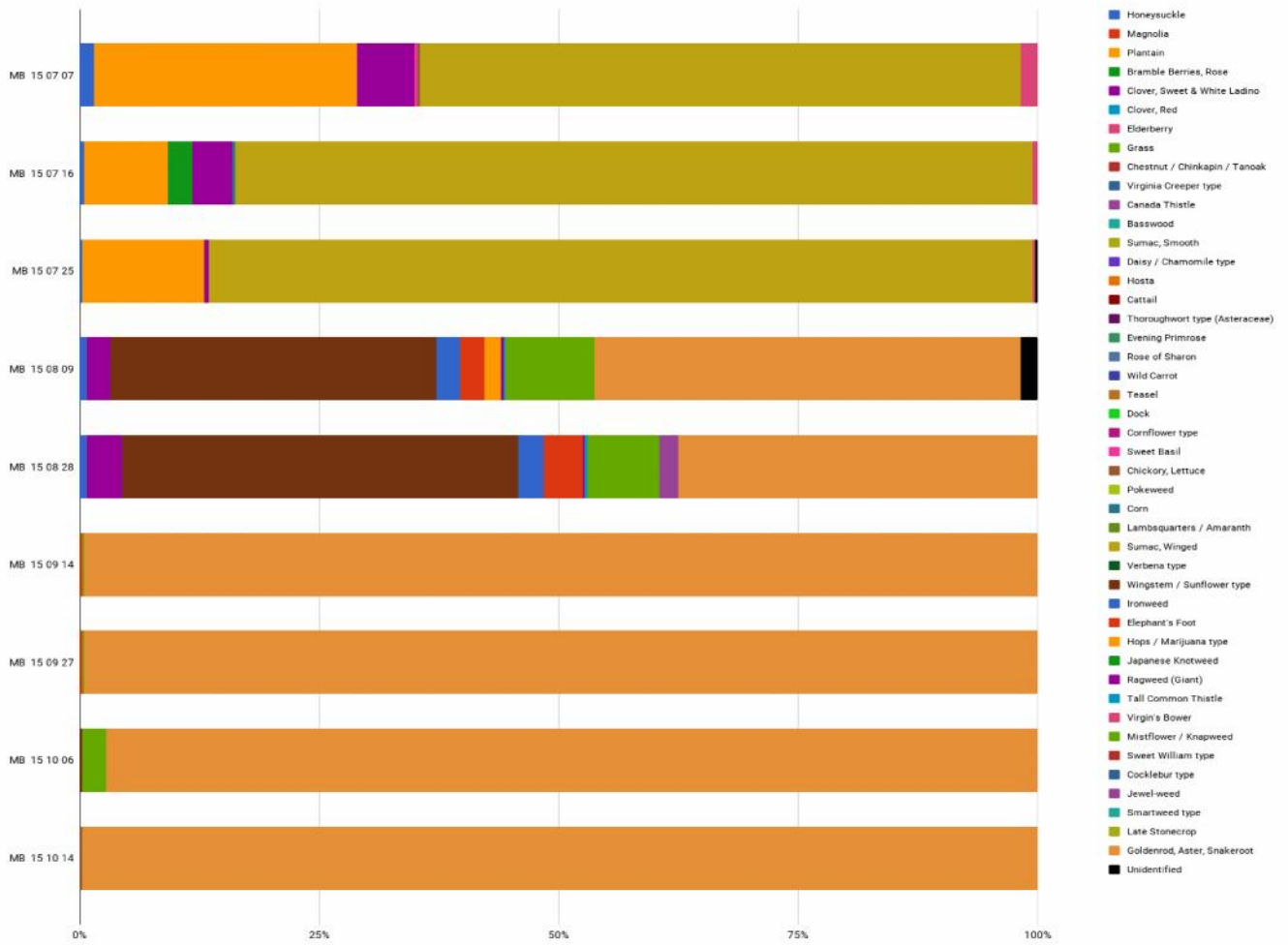
Quiet Dell: July-October 2015 Percentages



Clarksburg July-October 2015 Percentages



Jackson County July-October 2015 Percentages

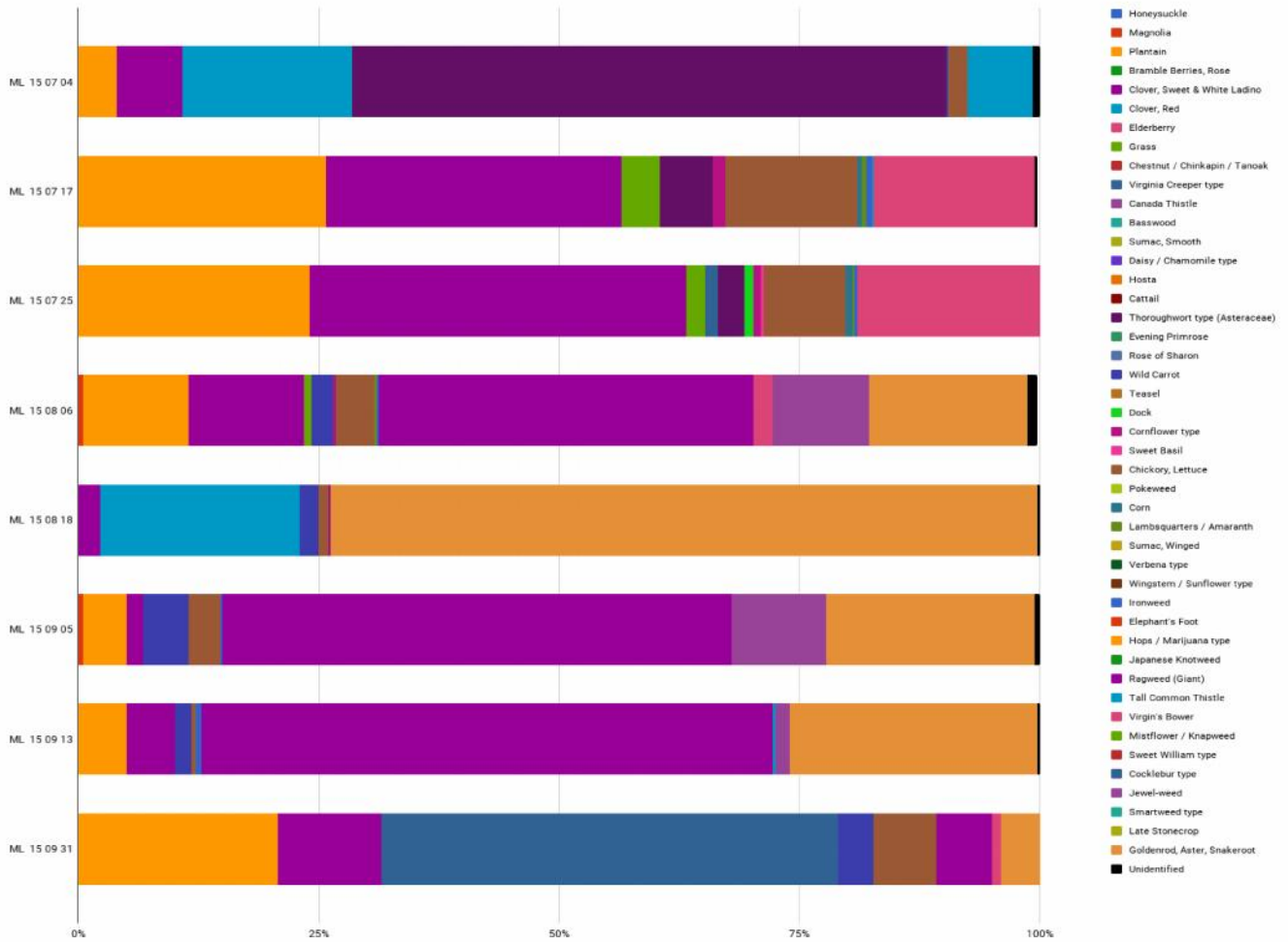


Percentages of each pollen type by date, March through June, rounded to nearest 1%. Plant list organized by approximate bloom time.

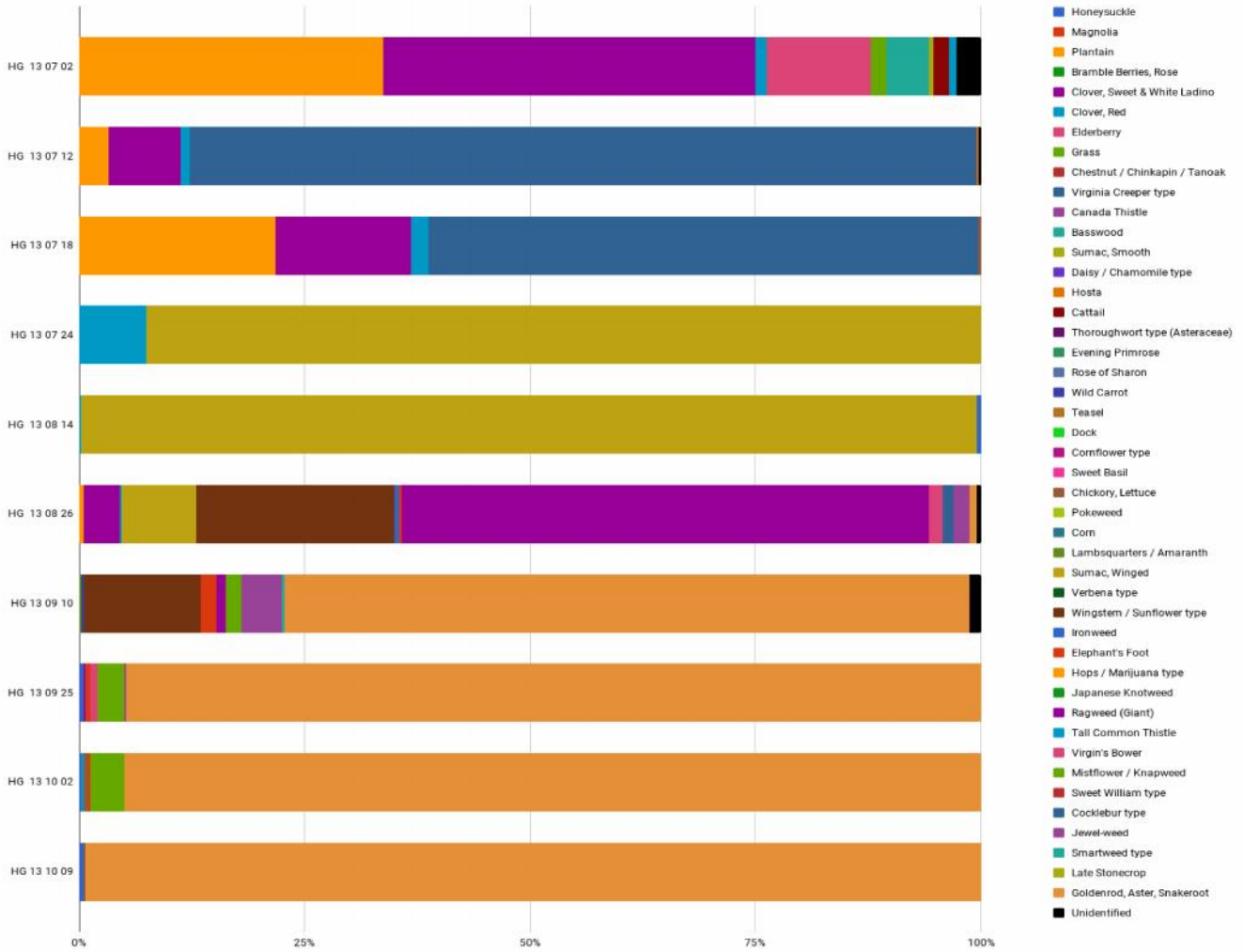
Near Cool Ridge WV, Raleigh County. 3000 ft. elevation, about 37.636 latitude.

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
Lonicera	Honeysuckle	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
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%
Rubus / Rosa	Bramble Berries, Rose	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	27 7%	123 31%	157 39%	48 12%	9 2%	7 2%	20 5%	43 11%
Trifolium pretense	Clover, Red	71 18%	0 0%	0 0%	0 0%	83 21%	0 0%	0 0%	0 0%
Sambucus	Elderberry	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Poaceae	Grass	0 0%	16 4%	8 2%	3 1%	0 0%	0 0%	0 0%	0 0%
Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Parthenococcus	Virginia Creeper type	0 0%	1 0%	5 1%	1 0%	0 0%	0 0%	0 0%	190 48%
Cirsium arvense	Canada Thistle	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Tilia	Basswood	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Rhus glabra	Sumac, Smooth	0 0%	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%
Hosta	Hosta	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Typha	Cattail	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	247 62%	22 6%	11 3%	0 0%	0 0%	0 0%	0 0%	0 0%
Oenothera, likely speciosa	Evening Primrose	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
Hibiscus syriacus	Rose of Sharon	0 0%	0 0%	0 0%	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%
Dipsacus fullonums	Teasel	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%
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%

Raleigh County July-October 2015 Percentages

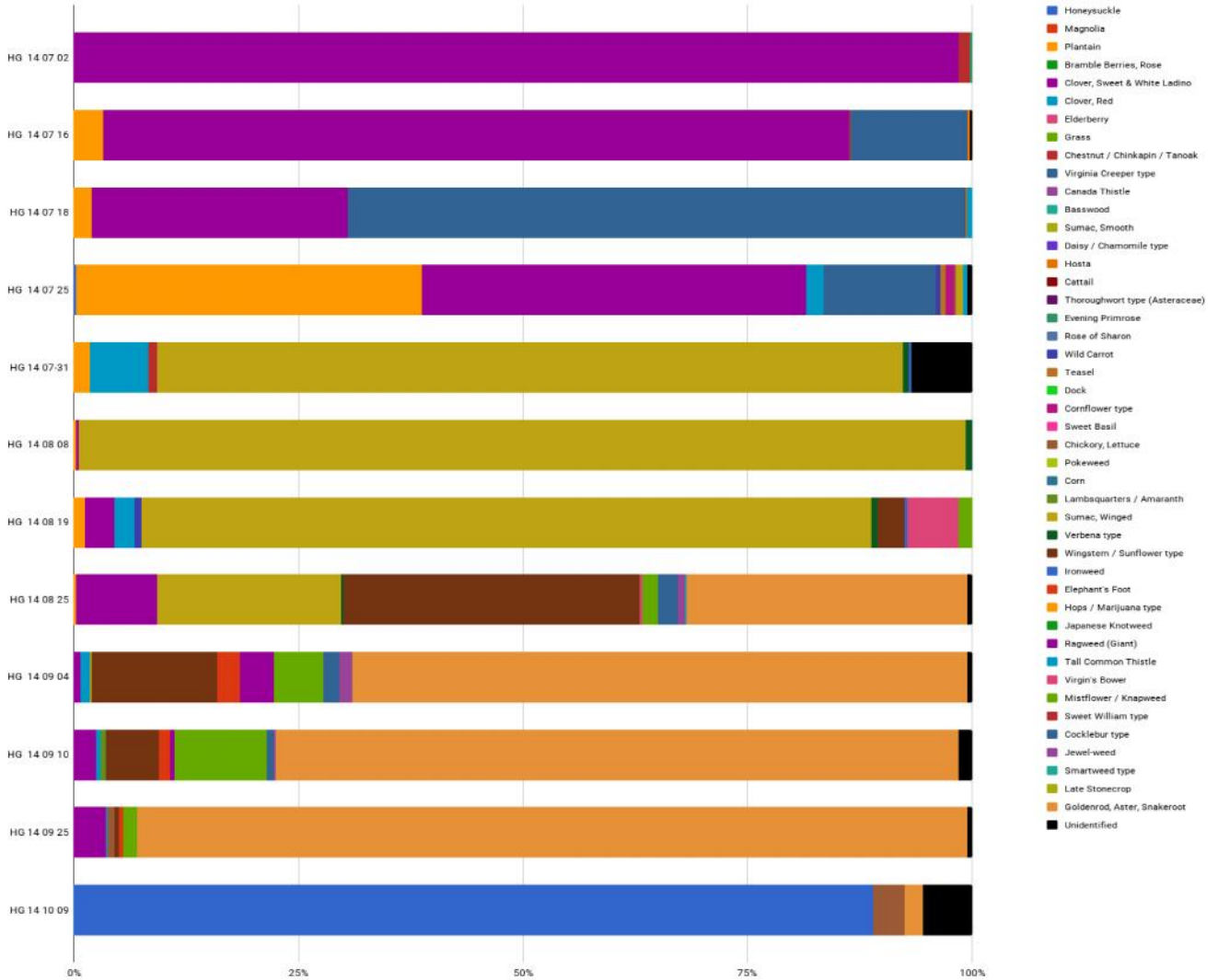


Salem July-October 2013 Percentages

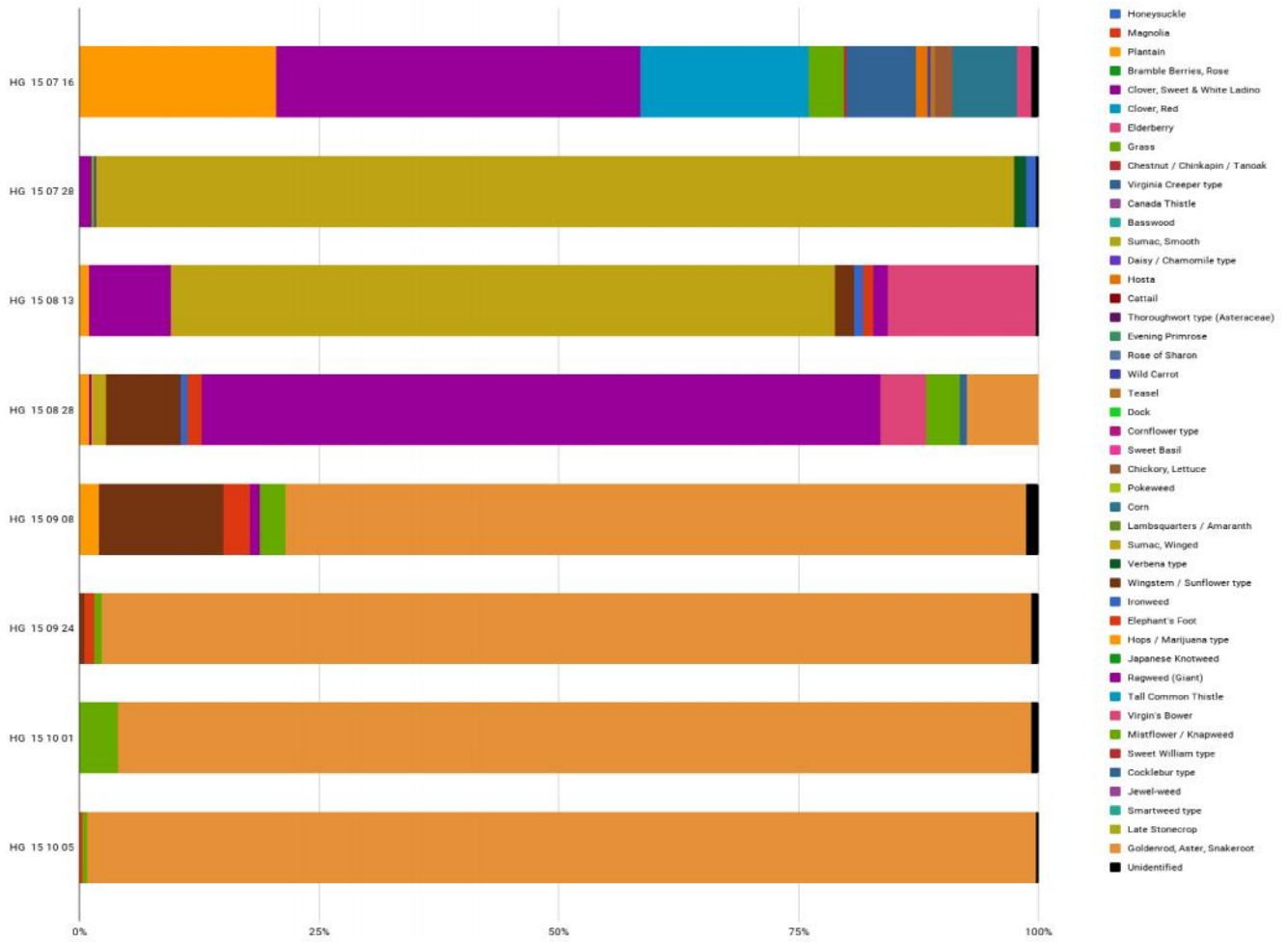


Scientific Name	Common Name	July 2		July 16		July 18		July 25		July 31		Aug. 8		Aug. 19		Aug. 25		Sept. 4		Sept. 10		Sept. 25		Oct. 9			
Ocimum basilicum	Sweet Basil	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Cichorium / Lactuca type	Chickory, Lettuce	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	3	1%	7	4%		
Phytolacca	Pokeweed	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Zea mays	Corn	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Chenopodium / Amaranth	Lambsquarters / Amaranth	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	2	1%	0	0%	0	0%
Rhus copallinum	Sumac, Winged	0	0%	0	0%	0	0%	3	1%	332	83%	395	99%	325	81%	82	21%	1	0%	0	0%	0	0%	0	0%	0	0%
Verbena type	Verbena type	0	0%	0	0%	0	0%	0	0%	3	1%	3	1%	3	1%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Verbesina / Helianthus	Wingstem / Sunflower type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	12	3%	132	33%	56	14%	24	6%	2	1%			0	0%
Vernonia	Ironweed	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Elephantopus	Elephant's Foot	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	10	3%	5	1%	2	1%		
Humulus, Can'bis	Hops / Hemp	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Polygonum cusp.	Jap. Knotweed	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Ambrosia	Ragweed (Giant?)	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	15	4%	2	1%	0	0%	0	0%
Cirsium altiss.	Common Thistle	0	0%	0	0%	2	1%	2	1%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Clematis virginiana	Virgin's Bower	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	23	6%	1	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%	6	2%	7	2%	22	6%	41	10%	6	2%			0	0%
Dianthus type	Sweet William	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Xanthium type	Cocklebur type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	9	2%	7	2%	3	1%	0	0%	0	0%	0	0%
Impatiens capensis	Jewel-weed	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	3	1%	6	2%	1	0%	0	0%	0	0%	0	0%
Persicaria.	Smartweed type	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Sedum	Late Stonecrop	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	125	31%	274	69%	304	76%	370	93%	4	2%		
	Unidentified	0	0%	1	0%	0	0%	2	1%	27	7%	0	0%	0	0%	2	1%	2	1%	6	2%	2	1%	11	6%		
Salem 2014		400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	400	100%	200	100%

Salem July-October 2014 Percentages



Salem July-October 2015 Percentages



Month-By-Month Comments

July Comments:

Significant pollen types in the month of July included Clover, Tanoak, Plantain, Virginia Creeper, Chicory / Lettuce, Lambsquarters, Corn, Thoroughwort type, and Winged Sumac. The most universally significant July pollen types were Clover and Plantain. However when it came to quantity of pollen brought in, the maximum amount of Winged Sumac in a July sample was more than nine times that of clover. Usually, when Winged sumac was found in a sample, the volume of pollen brought in took a significant jump. The one exception to this was the Clarksburg samples in which the July 30 sample contained a high percentage of Winged Sumac, and yet the total pollen collected was very low.

Winged Sumac was not universally abundant. It was completely absent from the Raleigh County samples, insignificant in Quiet Dell, and also insignificant in the Clarksburg location in terms of quantity. However at the Salem location, where pollen was collected for three consecutive years, Winged Sumac turned out to be significant in all three years. This tells us that Winged Sumac can be a reliable and highly sought after by bees, and wider distribution of the plant could be useful. It was also very significant in the Jackson County samples.

English Plantain (*Plantago lanceolata*) appears to be a highly beneficial lawn plant for honey bees (and by field observation, for other wild pollinators as well).

Tanoak pollen grains closely resemble Chestnut, yet while Chestnut typically blooms in June, Tanoak blooms throughout the summer into August, so this would be the probable source of the pollen found in these samples. Tanoak is planted ornamentally.

The July 29 sample from Quiet Dell stands out as the only important occurrence of Lambsquarters / Amaranth type pollen, in which well over half of the sample came from this source. Very little total pollen was collected on this date. The project leader has observed and photographed honey bees working a patch of Lambsquarters (*Chenopodium album*) for pollen. However the pollen grains in this sample better matched the lower pore density of Amaranth, such as Redroot Pigweed (*Amaranthus retroflexus*). In the August 6 sample from Raleigh County, grains with both the lower and higher pore density were found, but only the grains with the higher density (matching *C. album*) were numerous enough to be found in the 400 grain count, while the Amaranthus type were extremely scarce. The difference between the two was not clear in the reference materials until the project leader had his own pollen pellet samples treated in December 2018, by which time it was too late to revise the tables and bar graphs to reflect this detail.

The most likely floral origin of the pollen that matched the Chicory / Lettuce references is still little bit mysterious. We know that bees will rarely be seen on Chicory. The pollen of this type showed up so often and sometimes in a high enough percentage that the project leader suspects another species with pollen grains very similar in appearance might be involved.

The appearance of a Rosaceae pollen type in a July sample and an August sample, long after most Rosaceae species have long finished blooming, could indicate the presence of the native Flowering

Raspberry (*Rubus odoratus*) which blooms through July and August. The project leader has watched honey bees gathering pollen from this plant at other locations in West Virginia. Another possibility would be domestic fall bearing / everbearing raspberries.

Corn pollen rarely appeared in the study, and Soybean pollen never did, reflecting the general absence of crop agriculture in most of West Virginia, due to its absence of flat ground. Only once did corn pollen appear in a significant percentage of a sample, at the Clarksburg location on July 22.

August Comments:

Significant pollen types in the month of August included Winged Sumac, Ragweed, Sunflower / Wingstem, Clematis, and Elephant's Foot.

The Sunflower / Wingstem type was an important source of pollen in every location except the Raleigh County site. Wingstem and Sunflower pollen are practically indistinguishable 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.

Once again, Winged Sumac outshined the other pollen sources. The highest amount collected in a single day was from an August sample collected at the Salem location, and was three-and-a-half times more than the highest amount of Sunflower / Wingstem pollen seen in any sample.

Ragweed pollen, being a low protein food source that alone cannot sustain bee colony health, ranked surprisingly high for both percentage of a sample, and amount of pollen collected in a sample. Common Ragweed and Giant Ragweed are two prevalent species in the region. The project leader has observed honey bees foraging vigorously on Giant Ragweed, but has yet to find bees making use of Common Ragweed, and suspects Giant Ragweed to be the primary source of the Ragweed pollen found.

Clematis pollen was present in samples from all locations except the Clarksburg site, and was most prevalent in a small August sample from Quiet Dell where it comprised 30% of the sample. Overall, Clematis was less significant as a pollen source than the project leader expected, but is also a nectar producer and should be considered a good bee forage plant.

Elephant's Foot pollen was a type the project leader was interested in. Honey bees appeared to work it vigorously, but it was unfamiliar to beekeepers and absent from the beekeeping literature. It was years before he was even able to identify the plant. Comprising nearly 20% of an August sample, Elephant's Foot is a native plant that could be recommended for inclusion in seed mixes for added variety.

Red Clover made up 20% of an August sample. Red Clover pollen grains are practically indistinguishable from Crimson Clover pollen grains, and perhaps other *Fabaceae* types. While the honey bee proboscis is usually too short to reach the nectar in Red Clover flowers, honey bees have been observed working Red Clover for both nectar and pollen.

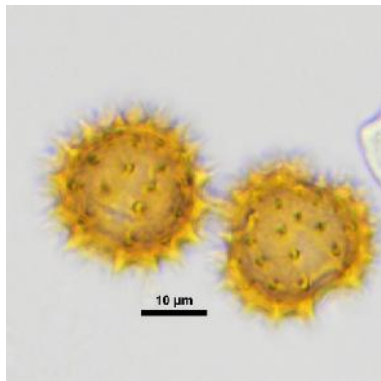
The sample containing the highest amount of Jewel Weed was an August sample from Raleigh County.

September Comments:

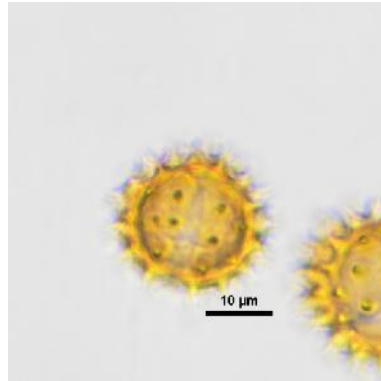
Significant pollen types in the month of September included the Goldenrod / Aster type, Ragweed, Wingstem, Virginia Creeper, and the Mistflower / Knapweed type.

One particularly unfortunate aspect of this study was the discovery that Snakeroot, Goldenrod, and Aster pollen grains are practically indistinguishable with light microscopy. The project leader 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 slight variations 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.

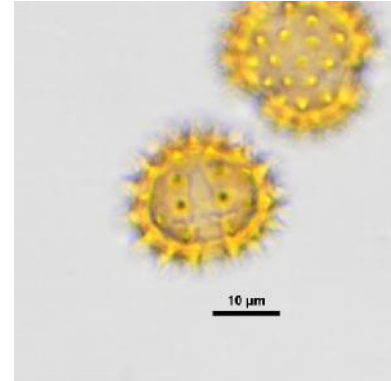
Below are some images of Snakeroot, Goldenrod, and Aster pollen grains showing their various features at different angles followed by photographs of bees on these flowers showing pollen pellet color.



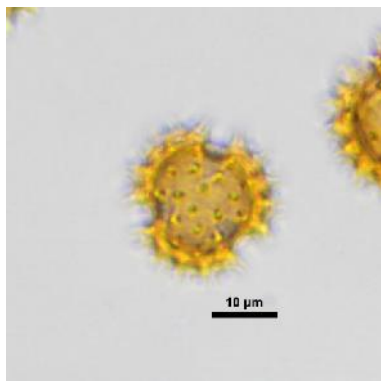
White Snakeroot (pore/furrow)



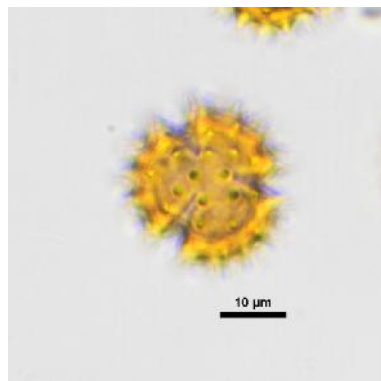
Tall Goldenrod (pore/furrow)



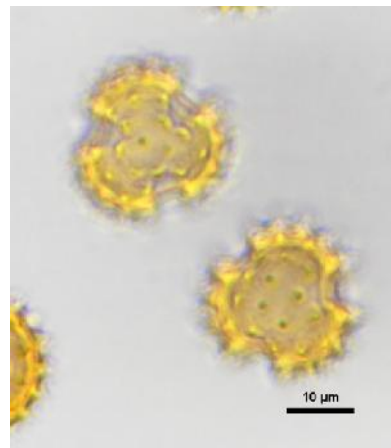
Late Aster (pore/furrow)



White Snakeroot (polar view)



Tall Goldenrod (polar view)



Late Aster (polar view)



Honey Bee on White Snakeroot



Honey Bee on Tall Goldenrod



Honey Bee on Fall Aster

It was striking to see the sudden change in pollen gathering when Goldenrod and Snakeroot began to come into bloom. The bees appear to have forgotten everything else in favor of one or more of these pollen types, and an increase in pollen gathering was seen almost universally. It could be argued that there is practically nothing else to be gathered at this time and that the bees might prefer other pollen types if they were as abundantly available, but the data we have suggest that honey bees find Fall Goldenrod and/or Snakeroot pollen attractive, or at least that it provides an abundant source of pollen.

The exception was the Raleigh County samples, in which the switch to the Goldenrod type was much less striking. The cause of this difference was not determined, whether it was a one-year incident due to something affecting the productivity of the Goldenrod plants overall, or a low quantity of the plants in the area, or some other factor. It is also very interesting to note that the “Goldenrod flow” in the Jackson County location appeared to begin unusually early and continued to the end of the season. This seems to indicate an additional plant type involved, with pollen of the same appearance, which began to bloom earlier than Goldenrod and Snakeroot.

As mentioned above, the two pollen types are very difficult to distinguish with light microscopy. However we can get a good lead 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 above). Based on the color of the pellets in practically all the September samples, it is obvious that Goldenrod dominates significantly over Snakeroot.

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 dioxide¹. 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 CO₂ 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>)

Parthenocissus (Virginia Creeper type) pollen making up nearly 50% of the Raleigh County September 31 sample was a real shocker. The project leader is unaware of any other plant type with pollen grains matching those of Parthenocissus, and September 31 is well outside of the normal bloom period for this plant.

The appearance of "Magnolia" pollen in August and September was another anomaly of the Raleigh County site. The timing of these samples being way out of Magnolia bloom season suggests there is another plant the bees were visiting with pollen grains nearly identical to "Magnolia". The closest possibility the project leader could find in his references was Yucca, which still did not match what was found in the samples perfectly.

Wild Carrot ("Queen Anne's Lace") is one of the most abundant wildflowers throughout the state, with a long bloom season through the summer when pollen is likely to be in short supply and most desperately needed by bees. The fact that it never contributed any major amount or percentage to any sample indicates that honey bees have a very low preference for it as a pollen source.

October Comments:

The only significant October pollen types were the Goldenrod / Aster / Snakeroot group and a single incidence of Honeysuckle.

There were no October samples collected at the Quiet Dell location, and the October samples collected in Raleigh county were lost due to a mishap, so the October samples came only from Salem (2013-2015), Jackson County, and Clarksburg.

It was interesting to see the Asteraceae type pollen grains in the October samples becoming more uniform in appearance as the bloom season drew to a close for the year. This was no surprise as Goldenrod and Snakeroot and most other plants die off, leaving Asters as the only significant plant type in bloom, and which do not seem to be damaged by frost. Two of the common Aster species at the Salem location are the lavender colored "Crooked Stem Aster" (*Symphotrichum prenanthoides*) and the small white "Calico Aster" (*S. lateriflorum*). Most abundant, however, is a large bushy white-flowered aster which could be "Frost Aster" (*S. pilosum*), and/or "Heath Aster" (*S. ericoides*), and/or "Panicked Aster" (*S. lanceolatum*) among others. The project leader did not take time to make definite identifications for the "White Bush Asters" common in the Salem sampling locality.

The high incidence of Honeysuckle pollen came from the Salem location in 2014. It was a small sample consisting of white pellets. This was so far outside the expected bloom period for Honeysuckle that a mistake might be suspected were it not that small amounts of Honeysuckle pollen were also collected from the same location in September and October of the previous year (but not in 2015). It is also interesting to note that in the Jackson County location, Honeysuckle was found in small amounts throughout the summer in the July and August samples. To date the most plausible explanation would be that the bees found and worked on Japanese Honeysuckle which has long bloom period and could potentially bloom or re-bloom throughout the summer and fall until frost.

One other plant known to bloom abundantly in the month of October is Smartweed (*Genus Persicaria*). The project leader has observed many bees working large patches of Smartweed and found it rare to see a honey bee collecting pollen from this plant. This study confirmed that what little pollen may end up in the hive from this plant could be accidental and incidental.

Variation between locations:

We wondered if in fact the plants bees foraged upon differed significantly from one location to the next, or if the bees' ability to forage widely would tend to have a moderating and equalizing effect. The two nearest locations in this study were more than 4 miles apart as the crow flies, so this study was not able to investigate this question in detail. The data collected however does confirm the former, that in a naturally diverse landscape, there are significant differences in foraging even between two fairly close



locations. Reasons for this are still speculative, likely due to significant differences in forage from location to location and the tendency of honey bees to forage closer to home when food is abundant. The topography of our area may also significantly affect foraging behavior, as hills and valleys probably tend to channel bees into certain areas. As noted in the Materials and Methods section above, even two colonies at the same location can bring in significantly different ratios of the primary pollen types.

Specific differences between locations can be seen by comparing the tables and bar graphs above. Some interesting differences, as well as similarities, were noted in the Month-by-month comments section, above.

Additional Lists Described in Grant Proposal

A few lists were described in the Methods and Measurements section of the Grant Proposal that would be compiled using the data collected.

The plants utilized during shortages of pollen (shortages shown by low amounts of pollen trapped) can be found by comparing the Average Pollen Intake timelines with the Pollen Percentage Tables. They include late season Asters, Amaranth, Centaurea, the Chicory/Lettuce type, Clematis, Clover, Corn, Grass, Honeysuckle, Ironweed, Plantain, Ragweed, Sumac, Tanoak, Teasel, Virginia Creeper, and Wild Carrot.

The plants that at any time comprised over 45% of the bees' pollen intake are considered "important" and can be seen easily in the Pollen Importance Tables. They include (in order of highest to lowest percentage) the Goldenrod / Aster / Snakeroot type, Sumac, Clover, Honeysuckle, Virginia Creeper, Corn, Ragweed, a summertime Asteraceae "Thoroughwort" type, and Lambsquarters. Wingstem came close to being included, with its highest percentage at 41.25%, and its actual volume was estimated to be greater than that of Virginia Creeper. Plantain also came fairly close at 38.5% and an actual volume greater than that of Clover.

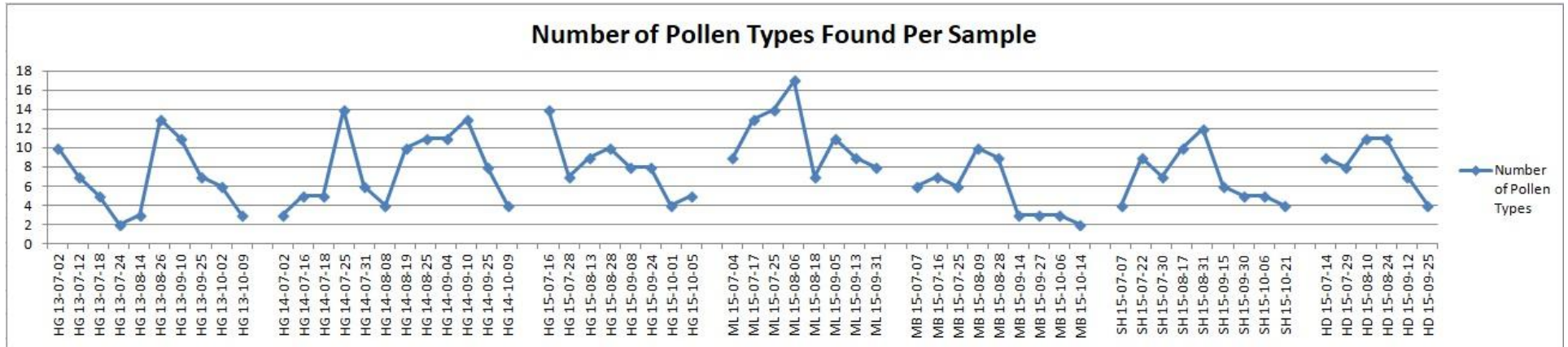
Plants unique to certain areas can be found by comparing the pollen percentage tables. The occurrence of Honeysuckle pollen was unique to the Salem and Jackson County locations. The "Magnolia" type was unique to Raleigh County. Pokeweed and Sweet William were unique to Clarksburg. The Hops/Hemp type was unique to Jackson County. The high occurrence of Amaranth pollen was unique to the Quiet Dell location, and the high amount of Corn to Clarksburg. Traces of Tanoak pollen were found in multiple locations, but only attained secondary status at Quiet Dell. Virginia Creeper was important at the Salem location in 2013 and 2014, but not in 2015. Hosta pollen was only found in the Salem samples, in trace amounts all three years. Verbena and Smartweed were also unique to Salem. The summertime Asteraceae "Thoroughwort" type was only important at Raleigh county, and virtually absent elsewhere.

Plants universally important can also be found by comparing the pollen percentage tables. The two types comprising important percentages universally (at all locations) were clover and the Goldenrod / Aster / Snakeroot type. Plantain pollen was found at all locations, often contributing to a considerable degree. Wingstem contributed considerably to all locations except Raleigh County.

The level of diversity of plant types present in a sample is relative. The average number of pollen types found in all the analyzed samples combined was about 7.66. There were eight samples in which 3 or fewer types were found, and eight samples in which 12 or more types were found. The chart below shows the number of pollen types found in all 62 samples analyzed from each location. The vertical axis represents the number of different pollen types found, with the samples listed across the horizontal axis.

As should be expected, periods of low diversity tended to occur when fewer species were blooming, but also occurred when a single species provided a superabundant supply of pollen, such as Winged Sumac. There was a general pattern of higher pollen diversity in the August samples compared to the other

months. Across all locations, October showed the lowest average pollen diversity at 4 types. July was slightly above average at 7.73. September was above average at 7.93, and August showed the highest average diversity of pollen at 9.8 types per sample.



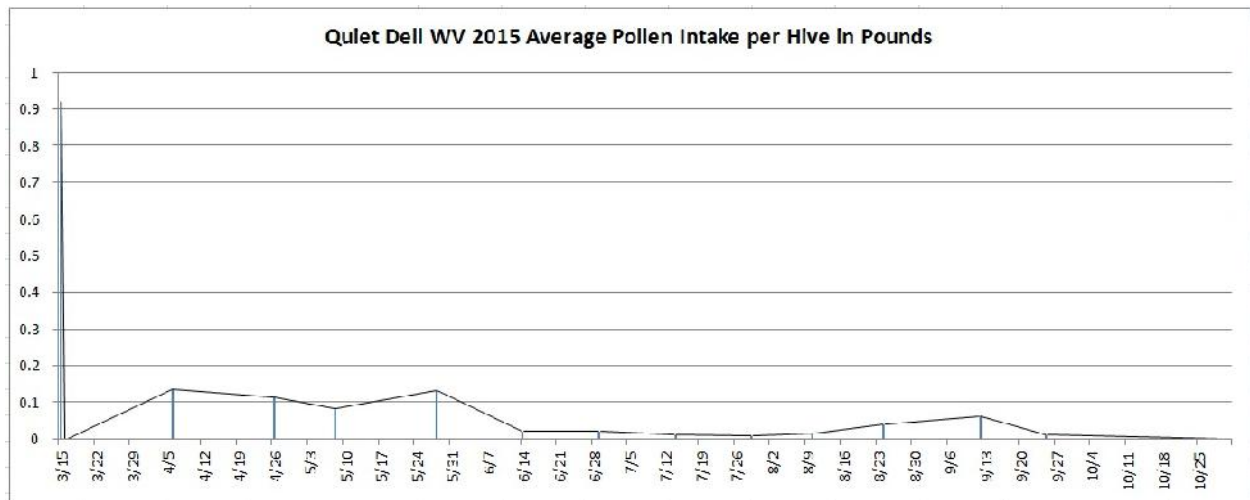
Average Pollen Intake per Hive in Pounds

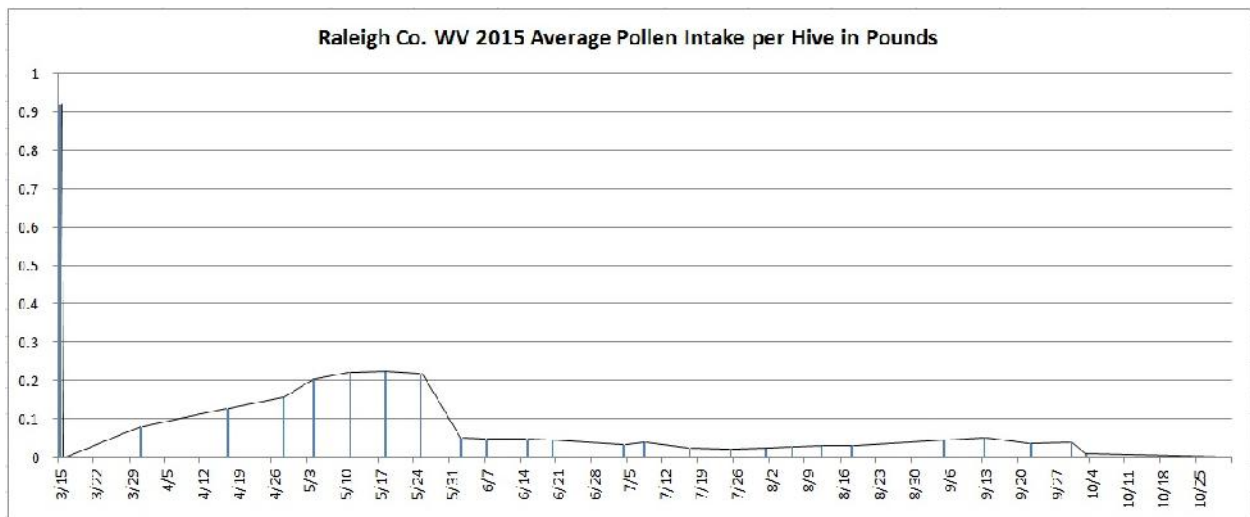
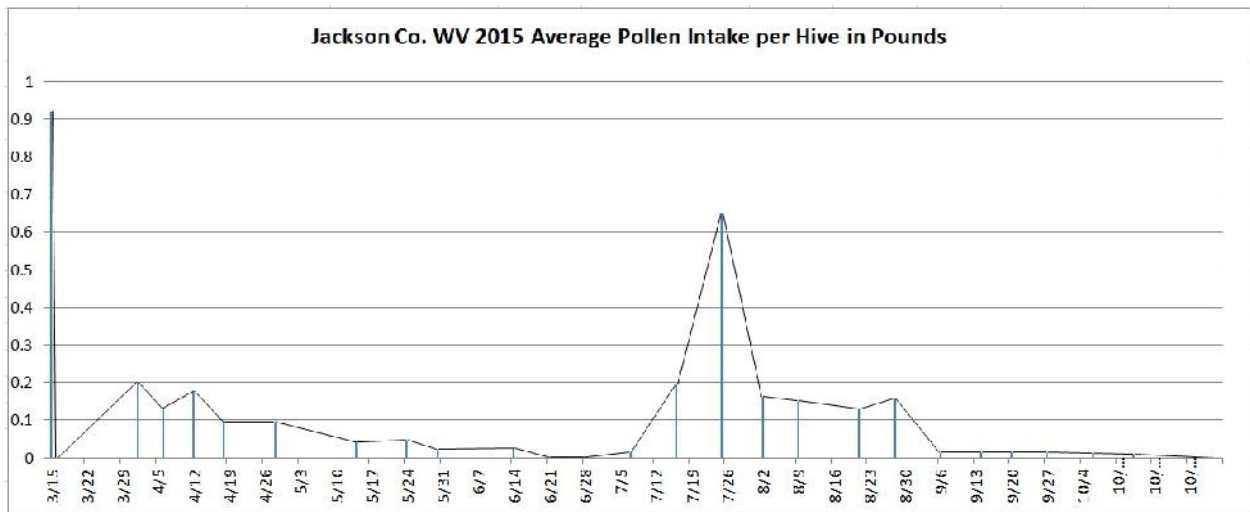
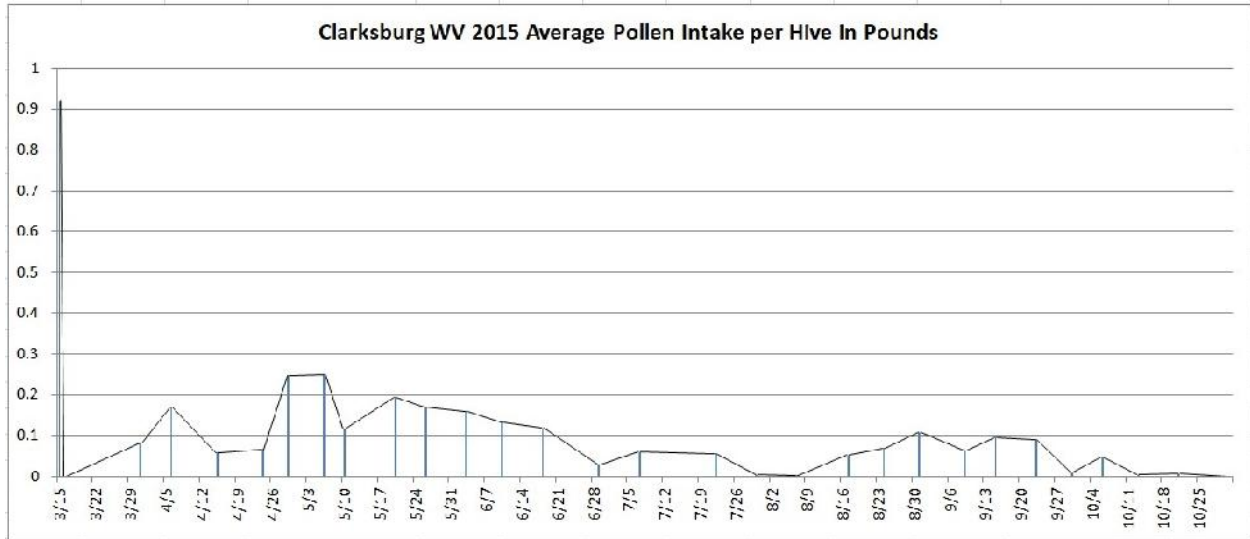
Below is a series of graphs showing the amount of pollen brought in through the year by weight, with a different graph for each location. It is important to remember that the pollen was collected on favorable foraging days, which can be scarce at times due to unfavorable weather. The true average pollen intake therefore may be lower than the connecting lines displayed on the graph. It is also possible that pollen collection could have spiked higher at points between collection dates.

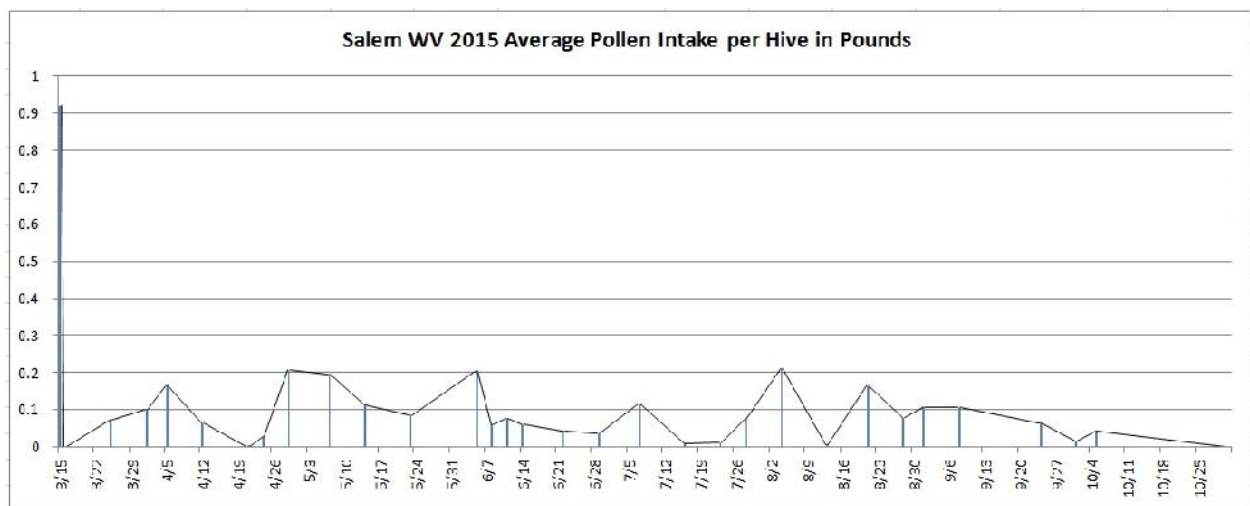
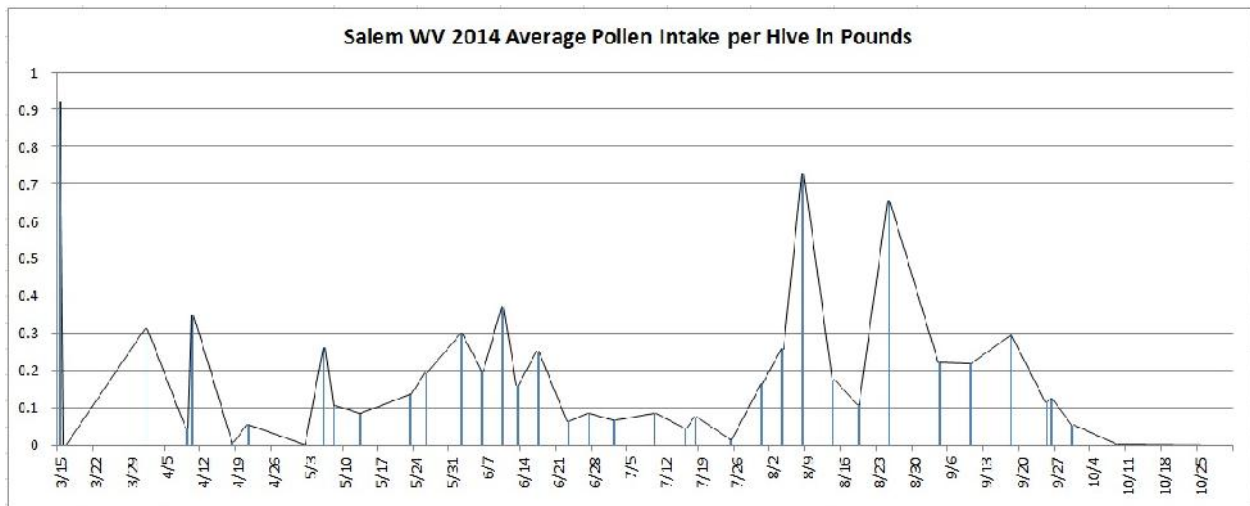
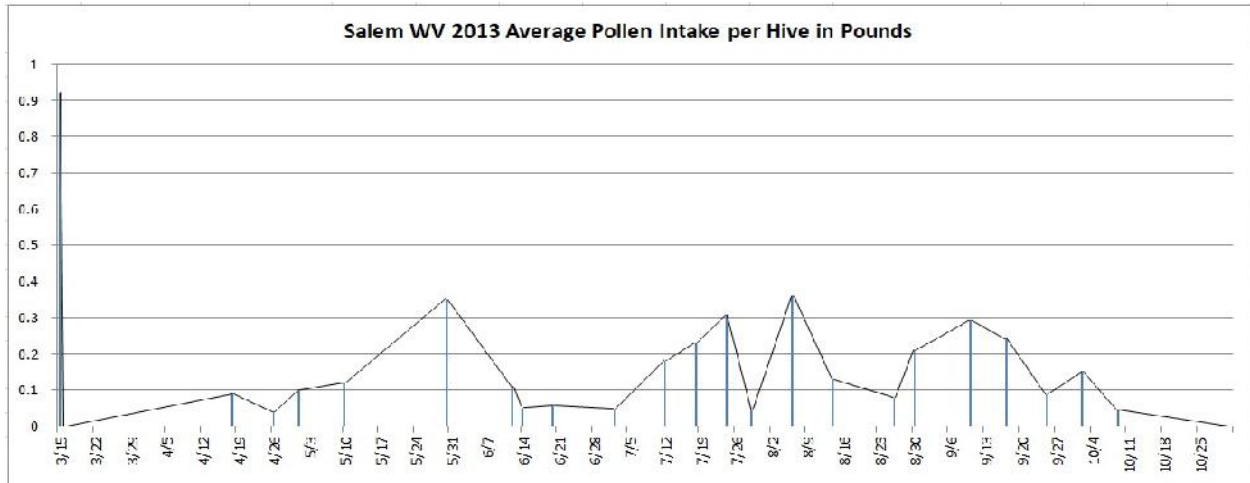
The vertical axis in the graphs is weight in pounds. The highest mark is one pound, about the maximum that could be collected in one day in our area with this method of collection. Continuous trapping would stimulate the bees to compensate by collecting much larger amounts. Collecting in short single-day periods helped minimize this behavior so that our data would better represent the natural foraging behavior.

The dates at the bottom show 1-week intervals from March to October, while the vertical lines show points at which samples were taken. The height of the vertical lines shows the amount of pollen collected in that sample, corresponding to the weights on the vertical axis. *(Ignore the high vertical line on the left which was used to create a uniform chart between all locations.)*

If more information was known about the protein content of each pollen type, a similar chart could be compiled showing the amount of actual protein being gathered by the bees at each point through the active season.







Pollen Importance Tables, July-October

Pollen Type Importance to Honey Bees, by % of total daily intake (TDI) and Weight Organized by maximum percentage found in any sample. Types found July through October, West Virginia 2013-2015, Northeast SARE FNE17-882 * max % and max wt in different samples				
Scientific Name	Common Name	Max % of TDI	Max weight /hive /day, lbs	
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	99.75	0.2223	*
Rhus copallinum	Sumac, Winged	99.25	0.71851	*
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	98.5	0.0656	
Lonicera	Honeysuckle	89	0.00255	
Parthenococcus	Virginia Creeper type	87.25	0.15705	
Zea mays	Corn	82	0.04442	
Ambrosia	Ragweed (Giant)	70.75	0.05536	
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	61.75	0.02025	
Chenopodium/Amaranth	Lambsquarters / Amaranth	58.75	0.00459	
Verbesina / Helianthus	Wingstem / Sunflower type	41.25	0.21656	*
Plantago	Plantain	38.5	0.08288	*
Cichorium / Lactuca type	Chickory, Lettuce	31.25	0.04	
Clematis virginiana	Virgin's Bower	30	0.01133	*
Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	27.25	0.00213	*
Trifolium pratense	Clover, Red	20.75	0.02295	*
Elephantopus	Elephant's Foot	19.25	0.00752	
Sambucus	Elderberry	11.5	0.00546	
Conoclinium / Centaurea type	Mistflower / Knapweed type	10.25	0.02226	
Impatiens capensis	Jewel-weed	10	0.01316	*
Rumex	Dock	7.25	0.00785	
Rubus / Rosa	Bramble Berries, Rose	6.75	0.00488	*
Cirsium altissimum	Tall Common Thistle	6.75	0.00221	
Tilia	Basswood	4.75	0.00226	
Apiaceae (i.e. Daucus)	Wild Carrot	4.75	0.00218	
Vernonia	Ironweed	4.75	0.00434	*
Chrysanthemum / Matricaria	Daisy / Chamomile type	4.5	0.00028	

Poaceae	Grass	4	0.00094	
Dipsacus fullonums	Teasel	2.75	0.00026	*
Xanthium type	Cocklebur type	2.25	0.01477	
Typha	Cattail	1.75	0.00083	
Humulus / Cannabis type	Hops / Hemp type	1.75	0.00265	
Dianthus	Sweet William type	1.75	0.00048	*
Hosta	Hosta	1.25	0.00045	*
Centaurea cyanus type	Cornflower type	1.25	0.00029	
Verbena type	Verbena type	1.25	0.00546	*
Phytolacca	Pokeweed	0.75	0.00003	
Magnolia	Magnolia	0.5	0.00023	
Rhus glabra	Sumac, Smooth	0.5	0.00024	
Oenothera, likely speciosa	Evening Primrose	0.25	0.00017	
Ocimum basilicum	Sweet Basil	0.25	0.00006	
Persicaria	Smartweed type	0.25	0.00164	
Cirsium arvense	Canada Thistle	0	0	
Hibiscus syriacus	Rose of Sharon	0	0	
Fallopia japonica	Japanese Knotweed	0	0	
Sedum	Late Stonecrop	0	0	

Pollen Type Importance to Honey Bees, by % of total daily intake (TDI) and Weight

Organized by maximum weight collected per colony per day.

Types found July through October, West Virginia 2013-2015, Northeast SARE FNE17-882

* max % and max wt in different samples

Scientific Name	Common Name	Max % of TDI	Max weight /hive /day, lbs	
Rhus copallinum	Sumac, Winged	99.25	0.71851	*
Solidago / Aster / Ageratina.	Goldenrod, Aster, Snakeroot	99.75	0.2223	*
Verbesina / Helianthus	Wingstem / Sunflower type	41.25	0.21656	*
Parthenocussus	Virginia Creeper type	87.25	0.15705	
Plantago	Plantain	38.5	0.08288	*
Melilotus & Trifolium repens	Clover, Sweet & White Ladino	98.5	0.0656	
Ambrosia	Ragweed (Giant)	70.75	0.05536	
Zea mays	Corn	82	0.04442	
Cichorium / Lactuca type	Chickory, Lettuce	31.25	0.04	
Trifolium pretense	Clover, Red	20.75	0.02295	*
Conoclinium / Centaurea type	Mistflower / Knapweed type	10.25	0.02226	
Eupatorium type (Asteraceae)	Thoroughwort type (Asteraceae)	61.75	0.02025	
Xanthium type	Cocklebur type	2.25	0.01477	
Impatiens capensis	Jewel-weed	10	0.01316	*
Clematis virginiana	Virgin's Bower	30	0.01133	*
Rumex	Dock	7.25	0.00785	
Elephantopus	Elephant's Foot	19.25	0.00752	
Sambucus	Elderberry	11.5	0.00546	
Verbena type	Verbena type	1.25	0.00546	*
Rubus / Rosa	Bramble Berries, Rose	6.75	0.00488	*
Chenopodium/Amaranth	Lambsquarters / Amaranth	58.75	0.00459	
Vernonia	Ironweed	4.75	0.00434	*
Humulus / Cannabis type	Hops / Hemp type	1.75	0.00265	
Lonicera	Honeysuckle	89	0.00255	
Tilia	Basswood	4.75	0.00226	
Cirsium altissimum	Tall Common Thistle	6.75	0.00221	
Apiaceae (i.e. Daucus)	Wild Carrot	4.75	0.00218	

Castanea / Notholithocarpus	Chestnut / Chinkapin / Tanoak	27.25	0.00213	*
Persicaria	Smartweed type	0.25	0.00164	
Poaceae	Grass	4	0.00094	
Typha	Cattail	1.75	0.00083	
Dianthus	Sweet William type	1.75	0.00048	*
Hosta	Hosta	1.25	0.00045	*
Centaurea cyanus type	Cornflower type	1.25	0.00029	
Chrysanthemum / Matricaria	Daisy / Chamomile type	4.5	0.00028	
Dipsacus fullonums	Teasel	2.75	0.00026	*
Rhus glabra	Sumac, Smooth	0.5	0.00024	
Magnolia	Magnolia	0.5	0.00023	
Oenothera, likely speciosa	Evening Primrose	0.25	0.00017	
Ocimum basilicum	Sweet Basil	0.25	0.00006	
Phytolacca	Pokeweed	0.75	0.00003	
Cirsium arvense	Canada Thistle	0	0	
Hibiscus syriacus	Rose of Sharon	0	0	
Fallopia japonica	Japanese Knotweed	0	0	
Sedum	Late Stonecrop	0	0	

Findings related to Beekeeper Profitability:

Pollen income is one of the important foundations of colony strength, and it is colony strength that makes beekeeping profitable.

The graphs of Average Pollen Intake demonstrate that a period of low pollen intake is indeed common in the summer, starting as early as June in some cases and sometimes extending into August. Low pollen intake in the summer months is no doubt involved with hive population dynamics as well as the disease susceptibility of individual bees (Transcriptional markers of sub-optimal nutrition in developing *Apis mellifera* workers - Corby-Harris et. al. 2014)

Keeping the above in mind, consider that arguably the biggest hindrance to beekeeper profitability is the parasitic mite *varroa destructor* which grows in population along with a colony's massive spring brood production. If the effect of reduced pollen forage later in the summer is both reduced brood production and reduced vitality of individual bees, then it is easy to see how the nutrition deficit and increasing mite issues compound one another to spiral a colony into decline.

Colony losses related to poor fall forage were widely reported throughout West Virginia in the year 2017 by both commercial and small scale beekeepers.

Finding ways to keep colony nutrition high is a common sense basic first line of defense. This study shows the pollen types available to bees during periods when pollen income is often low (i.e. July) and indicates the types of plants that could be used to increase the available forage at that time. Awareness of this dearth can help beekeepers understand colony dynamics and improve colony management.

From the data gathered it looks like any apiary would benefit highly from a large patch of Winged Sumac (*Rhus copallinum*). Sweet Clover (*Melilotus*) is another plant eagerly utilized by bees for pollen which could be used to improve the quantity of summer forage well into the month of July, especially White Sweet Clover which tends to bloom a little later than Yellow Sweet clover. Wingstem in particular (*Verbesina*), as well as Giant Ragweed (*Ambrosia*), Elephant's Foot (*Elephantopus*), and Lambsquarters (*Chenopodium album*), although they may not support colony growth individually, could add beneficial variety and be eagerly worked by bees. Tanoak (*Notholithocarpus*) and Virginia Creeper (*Parthenocissus*) are options very much worthy of further investigation. The nutritional quality of many of these is unknown to us at this point.

Conclusion

The study was successful in giving beekeepers in our area a fact-based picture of what their bees' pollen income looks like through the second half of the active season in an easy-to-understand format. We also demonstrated an effective method of establishing the facts.

Future Recommendations

The procedures followed in this investigation were effective in producing the desired data. If at any time a beekeeper or group of beekeepers desires to know the facts regarding the pollen forage of honey bees in their area, following the same procedures as for this project could be recommended. It is important to recruit individuals committed to regular pollen collection and record-keeping according to the guidelines, as well as an individual with experience in pollen grain identification and access to the required reference materials and microscopic equipment.

Pollen grain identification can be challenging and quality references are essential. Even with a high quality Nikon microscope and camera, the distinction between certain fall Asteraceae species was not possible, such as between Goldenrod, Aster, and White Snakeroot, or between Wingstem and regular Sunflowers. There is hope that Metabarcoding techniques could become a reliable method of quantitative pollen analysis that would circumvent the obstacles of visual microscopic analysis. If metabarcoding is successful at identifying the pollen types in a sample but unable to determine the quantity of each type, microscopic quantitative analysis would still be required.

There remains a continued need for more complete reference materials for light microscopy. Although references exist for some species in most of the common genera, there were numerous species common in our region for which we had no images, poor quality images, or only a single image from a single angle. July-to-October-blooming species for which more reference photos would be helpful include American Burnweed (*Erechtites hieraciifolius*), Hosta (*Hosta*), Rose Pink (*Sabatia angularis*), Japanese Knotweed (*Fallopia japonica*), Spotted Knapweed (*Centaurea maculosa*), Elephant's Foot (*Elephantopus tomentosus*), White Snakeroot (*Ageratina altissima*), and Ragweeds (*Ambrosia trifida*, *A. artemisiifolia*).

The SEM images on paldat.com were often helpful.

The volume information in the Pollen Importance Tables has limited accuracy at present. These calculations were made for each sample based simply on the pollen grain count and resulting percentages for each type, and the total weight of pollen collected in the sample. What the calculations did not consider was the sometimes drastic difference in individual pollen grain size. So, if the pollen grain count showed a smaller grain making up 50% of the sample, that pollen type would logically have made up less than 50% of the volume of the sample. Such differences in pollen grain volume were not taken into account when calculating the volume of each pollen type in a sample. The density of different pollen grains could also influence this calculation depending on whether actual weight or actual volume would be the more important factor to consider in a pollen's importance to honey bees.

The development of a comprehensive "field guide" similar to the classic wildlife field guides, which shows multiple SEM and Light Microscope images of the pollen grains at polar, oblique, and equatorial viewpoints, surface and cross-section, size of grains, bloom time of the species, and pointing out the differences between similar species, would be ideal.

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The mounting health issues of our technical advisor, Professor Bryant, including cancer, chemotherapy, and Acute Myeloid Leukemia are cause for much concern. When his earthly race is finished he will be sorely missed not only for his expertise, but also for his encouragement and generous support for projects such as this. It will be important to continue to be able to treat pollen samples for projects such as this for many years to come.

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