

Northeast SARE Farmer Grant 2015

Michael Staddon, Project Leader

Summary

The purpose of the West Virginia Pollen Project 2015 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. 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 by the project leader to provide a relative abundance of each pollen type present in the samples. Reports were compiled that showed the percentage of each pollen type in the March through June samples, and graphed the amount of pollen brought in by the bees through the whole year.

The results 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 nearly 100% of a sample, while at other times no one type made up more than 26%. Sometimes we saw exactly what we expected, such as Red Maple (*Acer rubrum*) in March, and at other times we saw very unexpected results such as a large percentage of Tupelo ("Black Gum" *Nyssa sylvatica*) in May. Challenges to the project included limited reference material for pollen identification, image quality when photographing pollen grains to help with identification, and the project leader's newness to pollen grain identification.

Introduction

In the project leader's attempts to manage honey bee colonies naturally, the issue of colony nutrition became much more important. Also, having been involved in beekeeper education since 2007, he became aware of misconceptions and many unanswered questions in the area of honey bee and plant relationships, especially in our 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 Tulip Poplar pollen only to discover later that it never comprises more than 2% of the honey bees' diet.

The hope of conducting pollen research was presented at local and state beekeepers meetings and interested beekeepers were recruited. Of the seven beekeepers initially committed to sampling pollen from their hives, five were able to followed through with submission of samples. Three were located in Harrison County, one in Jackson County, and one in Raleigh County at 3,000 feet elevation. We were



thrilled that Larry Campbell, who had previous experience with SARE grants, agreed to serve as technical advisor, especially for his input on the reporting.

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:

As soon as the grant was approved in February 2015, the needed pollen traps, plastic vials, and weighing scales were purchased and shipped to each collaborating beekeeper so that they could attach the pollen traps to their beehives and begin collecting pollen during the Maple flows in March.

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. This commitment was made prior to the submission of the grant proposal so that information on project expenses and estimated number of samples collected could be included in the grant proposal. 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 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 all night) 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 as planned except that two of the seven collaborators, representing three of the eight planned locations, were not able to submit any samples. 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. One of the collaborators 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:

80 of the submitted samples were chosen for analysis. The samples were chosen, based on the collection date, for evenly spaced dates - usually two samples per month for each collaborator for the months of March through July – so as to form an optimal picture of pollen gathering at regularly spaced dates through the season.

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 is 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 80 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.



Dr. Don Trisel of Fairmont State University provided a microscope loan for the project – a Micro III light microscope with a Leica EC3 camera mounted. The camera enabled photographs to be taken of the pollen grains and e-mailed to Professor Bryant if help was needed with identification. WVU also provided a loan of a VWR light microscope that offered an even clearer view of the pollen grains, but with no camera. There were no 60x objectives available so the project was conducted with the more common 100x

oil immersion and 40x objective lenses. These provided 1,000x and 400x magnifications. Although more time consuming, the project leader found oil immersion under the 100x lens essential for distinction between some of the pollen types and used this objective routinely. The 10x and 4x lenses were helpful



for finding locations on a slide more quickly when needed. Unfortunately we were not able to get images of sufficient quality with the Leica EC3 camera to reveal the morphology of the pollen grains in enough detail for definite identification of some of the more difficult pollen types. Using a Carson Hookupz universal adaptor attached to the WVU microscope with a 13 megapixel smart phone camera provided slight but insufficient improvement.

To conduct the analysis, 200 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 200 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), 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.

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 March and April 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. Some photographs of the pollen found were also included.

The analysis process took much longer than expected. Time demands during the busy April, May, and June beekeeping season also pushed the pollen analysis later than expected. Analysis of all the March

and April samples was finished in time to compile a power point presentation of the results-to-date which was delivered the at the West Virginia Beekeepers Association fall conference on September 25, 2015.

A late September reassessment of the project revealed that only the March-through-June prepared samples could be analyzed and reported on by year's end. It was agreed to include the July sample analysis with the analysis of the August-through-October samples in a future project, hopefully approved for 2016. Even with the adjustment, the project leader spent many more hours on the project than originally projected and provided for.

Analysis was conducted on the May and June samples and the last of the collaborator reports for those months was completed on December 9. The May-June collaborator report also included the whole-year graph of average pollen intake by weight based on the information recorded on the sample record sheets.

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.

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 March through June. 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. 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.

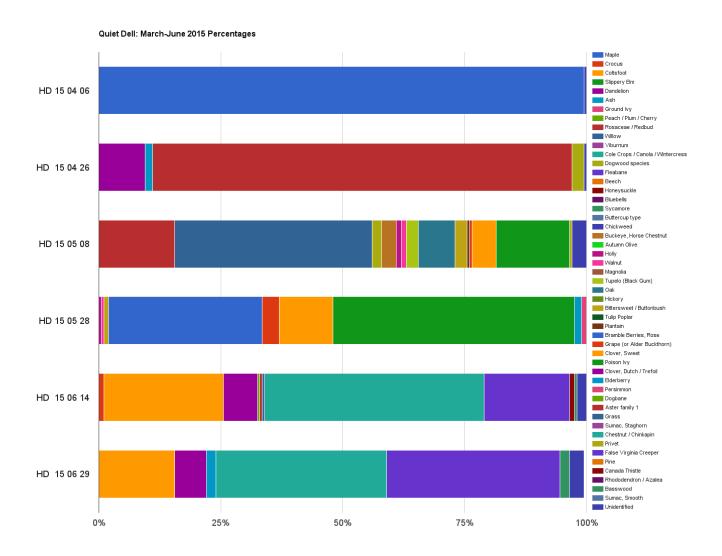
The **Pollen Importance Tables** organize the pollen types found by level of importance based on both highest percentage found in any sample, and on highest amount in weight gathered per hive at any one time. One table is organized in descending order based on percentage, the other is organized in descending 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 from all locations.

Pollen Percentage Tables and Bar Graphs: Percentages of each pollen type by date, March through June, 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 | Marcl | h/April | М | ay | Ju | ine |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | HD 15 04 06 | HD 15 04 26 | HD 15 05 08 | HD 15 05 28 | HD 15 06 14 | HD 15 06 29 |
| Acer | Maple | 100% | 0% | 0% | 0% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 0% | 10% | 0% | 0% | 0% | 0% |
| Fraxinus | Ash | 0% | 2% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 0% | 86% | 16% | 0% | 0% | 0% |
| Salix | Willow | 0% | 0% | 41% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 2% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 0% | 0% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 0% | 0% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 3% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 0% | 0% | 0% | 0% |
| llex | Holly | 0% | 0% | 1% | 1% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 1% | 1% | 0% | 0% |

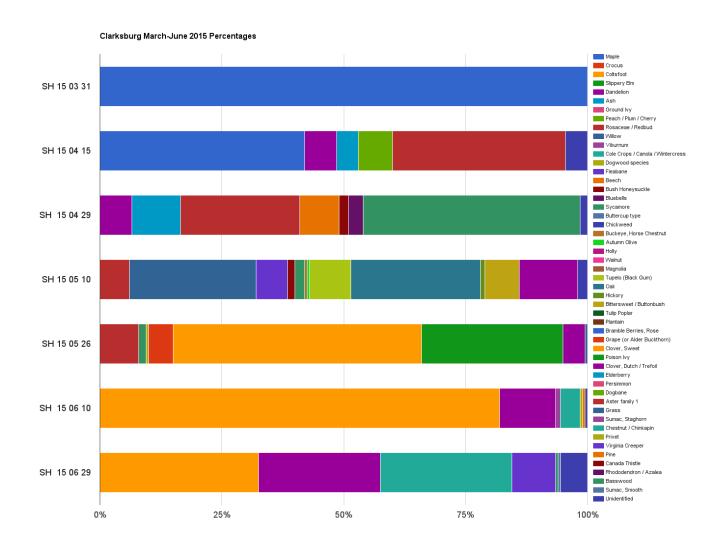
| Magnolia | Magnolia | 0% | 0% | 0% | 0% | 0% | 0% |
|-----------------------------|-----------------------------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 3% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 8% | 0% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 0% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 3% | 1% | 0% | 0% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 0% | 0% | 0% |
| Plantago | Plantain | 0% | 0% | 1% | 0% | 0% | 0% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 32% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 1% | 4% | 1% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 5% | 11% | 25% | 16% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 15% | 50% | 0% | 0% |
| Trifolium | Clover, Dutch / Trefoil | 0% | 0% | 0% | 0% | 7% | 7% |
| Sambucus | Elderberry | 0% | 0% | 0% | 2% | 0% | 2% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 1% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 1% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 1% | 0% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 1% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 45% | 35% |
| Ligustrum | Privet | 0% | 3% | 1% | 0% | 0% | 0% |
| Parthenocussus | False Virginia Creeper | 0% | 0% | 0% | 0% | 18% | 36% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 1% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 1% | 2% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% |
| | Unidentified | 1% | 1% | 3% | 0% | 2% | 3% |
| | | 100% | 100% | 100% | 100% | 100% | 100% |
| | | | | | | | |



South of Clarksburg WV, Harrison County. 1000 ft. elevation, about 39.226 latitude.

| Clarksburg | 2015 | | March/April | | М | ay | Ju | ne |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | SH 15 03 31 | SH 15 04 15 | SH 15 04 29 | SH 15 05 10 | SH 15 05 26 | SH 15 06 10 | SH 15 06 29 |
| Acer | Maple | 100% | 42% | 0% | 0% | 0% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 0% | 7% | 7% | 0% | 0% | 0% | 0% |
| Fraxinus | Ash | 0% | 5% | 10% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 7% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 0% | 36% | 25% | 6% | 8% | 0% | 0% |
| Salix | Willow | 0% | 0% | 0% | 26% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 0% | 7% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 8% | 0% | 0% | 0% | 0% |
| Lonicera | Bush Honeysuckle | 0% | 0% | 2% | 2% | 0% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 3% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 45% | 2% | 2% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| llex | Holly | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Junglans | Walnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

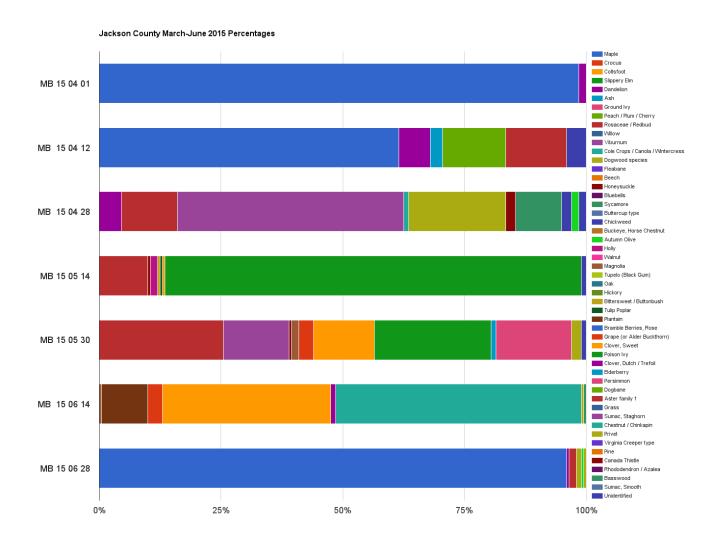
| Magnolia | Magnolia | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
|-----------------------------|-------------------------------|------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 0% | 9% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 0% | 27% | 0% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 0% | 7% | 1% | 0% | 0% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Plantago | Plantain | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 0% | 0% | 5% | 0% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 0% | 0% | 51% | 82% | 33% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 0% | 0% | 29% | 0% | 0% |
| Trifolium | Clover, Dutch / Trefoil | 0% | 0% | 0% | 12% | 5% | 12% | 25% |
| Sambucus | Elderberry | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 1% | 0% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 4% | 27% |
| Ligustrum | Privet | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Parthenocussus | Virginia Creeper | 0% | 0% | 0% | 0% | 0% | 0% | 9% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 0% | 1% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% | 1% |
| | Unidentified | 0% | 5% | 2% | 2% | 0% | 1% | 6% |
| | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |



East side of Ripley WV, Jackson County. 750 ft. elevation, about 38.820 latitude.

| Jackson County | 2015 | | March/April | | М | ay | Ju | ne |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | MB 15 04 01 | MB 15 04 12 | MB 15 04 28 | MB 15 05 14 | MB 15 05 30 | MB 15 06 14 | MB 15 06 28 |
| Acer | Maple | 99% | 62% | 0% | 0% | 0% | 0% | 96% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 2% | 7% | 5% | 0% | 0% | 0% | 1% |
| Fraxinus | Ash | 0% | 3% | 0% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 13% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 0% | 13% | 12% | 10% | 26% | 0% | 2% |
| Salix | Willow | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 47% | 0% | 14% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 1% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 20% | 0% | 0% | 0% | 1% |
| Erigeron | Fleabane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 2% | 1% | 1% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occinentalis | Sycamore | 0% | 0% | 10% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 2% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 2% | 0% | 0% | 0% | 1% |
| llex | Holly | 0% | 0% | 0% | 2% | 0% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

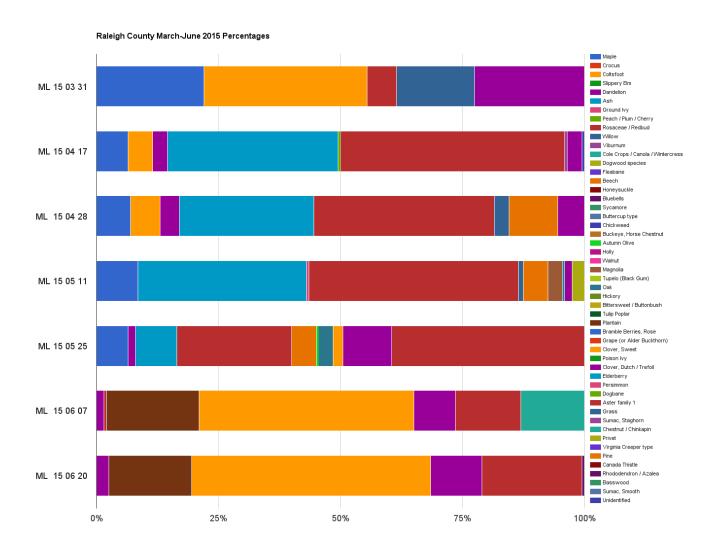
| Magnolia | Magnolia | 0% | 0% | 0% | 0% | 2% | 1% | 0% |
|-----------------------------|-------------------------------|------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Plantago | Plantain | 0% | 0% | 0% | 0% | 0% | 10% | 0% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 0% | 0% | 3% | 3% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 0% | 1% | 13% | 35% | 0% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 0% | 86% | 24% | 0% | 0% |
| Trifolium | Clover, Dutch / Trefoil | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Sambucus | Elderberry | 0% | 0% | 0% | 0% | 1% | 0% | 0% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 0% | 16% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 51% | 0% |
| Ligustrum | Privet | 0% | 0% | 0% | 0% | 2% | 1% | 1% |
| Parthenocussus | Virginia Creeper type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Unidentified | 0% | 4% | 2% | 1% | 1% | 0% | 0% |
| | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |



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

| Raleigh County | 2015 | | March/April | | М | ay | Ju | ine |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | ML 15 03 31 | ML 15 04 17 | ML 15 04 28 | ML 15 05 11 | ML 15 05 25 | ML 15 06 07 | ML 15 06 20 |
| Acer | Maple | 22% | 7% | 7% | 9% | 7% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 34% | 5% | 6% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 0% | 3% | 4% | 0% | 2% | 2% | 3% |
| Fraxinus | Ash | 0% | 35% | 28% | 35% | 9% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 1% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 6% | 46% | 37% | 43% | 24% | 1% | 0% |
| Salix | Willow | 16% | 0% | 3% | 1% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 1% | 0% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 10% | 5% | 5% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 0% | 0% | 1% | 0% | 0% |
| llex | Holly | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

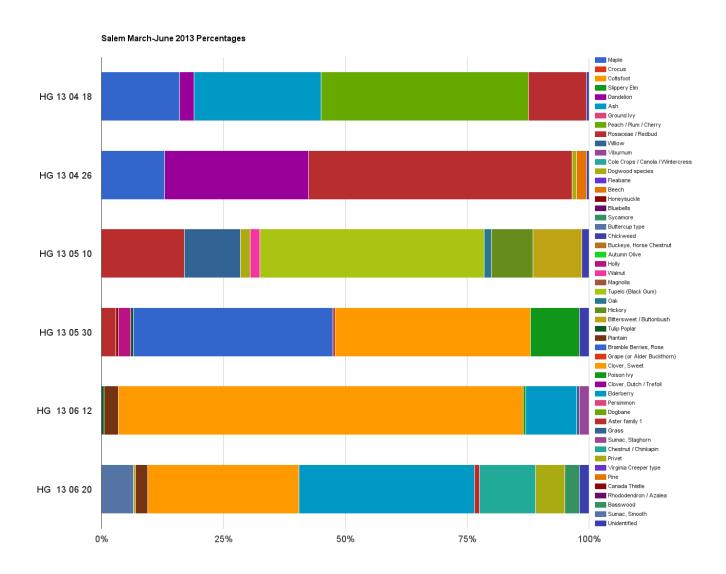
| Magnolia | Magnolia | 0% | 0% | 0% | 3% | 0% | 0% | 0% |
|-----------------------------|-------------------------------|------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 0% | 1% | 3% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Plantago | Plantain | 0% | 0% | 0% | 0% | 0% | 19% | 17% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 0% | 0% | 2% | 44% | 49% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Trifolium | Clover, Dutch / Trefoil | 23% | 3% | 6% | 2% | 10% | 9% | 11% |
| Sambucus | Elderberry | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 40% | 14% | 21% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 13% | 0% |
| Ligustrum | Privet | 0% | 0% | 0% | 3% | 0% | 0% | 0% |
| Parthenocussus | Virginia Creeper type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% | 1% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Unidentified | 0% | 1% | 0% | 0% | 0% | 0% | 0% |
| | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |



South of Salem WV, Harrison County. 1200 ft. elevation, about 39.263 latitude.

| Salem | 2013 | March | /April | М | ay | Ju | ne |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | HG 13 04 18 | HG 13 04 26 | HG 13 05 10 | HG 13 05 30 | HG 13 06 12 | HG 13 06 20 |
| Acer | Maple | 16% | 13% | 0% | 0% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 3% | 30% | 0% | 0% | 0% | 0% |
| Fraxinus | Ash | 26% | 0% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 43% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 12% | 54% | 17% | 3% | 0% | 0% |
| Salix | Willow | 0% | 0% | 12% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 1% | 2% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 2% | 0% | 0% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 0% | 1% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 7% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 0% | 0% | 0% | 0% |
| llex | Holly | 0% | 0% | 0% | 3% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 2% | 0% | 0% | 0% |

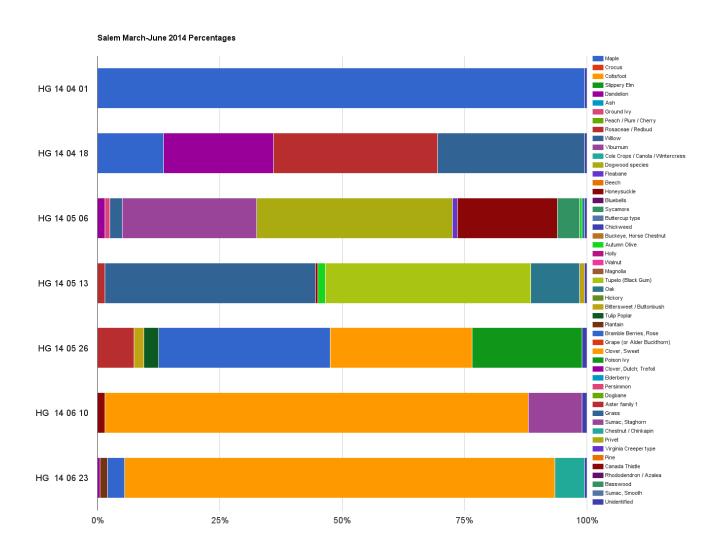
| Magnolia | Magnolia | 0% | 0% | 0% | 0% | 0% | 0% |
|-----------------------------|-------------------------------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 46% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 2% | 0% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 9% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 10% | 0% | 0% | 1% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 1% | 1% | 0% |
| Plantago | Plantain | 0% | 0% | 0% | 0% | 3% | 3% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 41% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 0% | 1% | 0% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 0% | 40% | 83% | 31% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 0% | 10% | 1% | 0% |
| Trifolium | Clover, Dutch / Trefoil | 0% | 0% | 0% | 0% | 0% | 0% |
| Sambucus | Elderberry | 0% | 0% | 0% | 0% | 11% | 36% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 0% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 0% | 1% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 1% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 2% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 12% |
| Ligustrum | Privet | 0% | 0% | 0% | 0% | 0% | 6% |
| Parthenocussus | Virginia Creeper type | 0% | 0% | 0% | 0% | 0% | 0% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 3% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% |
| | Unidentified | 1% | 1% | 2% | 2% | 0% | 2% |
| | | 100% | 100% | 100% | 100% | 100% | 100% |



South of Salem WV, Harrison County. 1200 ft. elevation, about 39.263 latitude.

| Salem | 2014 | Marcl | h/April | | May | | Ju | ine |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | HG 14 04 01 | HG 14 04 18 | HG 14 05 06 | HG 14 05 13 | HG 14 05 26 | HG 14 06 10 | HG 14 06 23 |
| Acer | Maple | 100% | 14% | 0% | 0% | 0% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 0% | 23% | 2% | 0% | 0% | 0% | 1% |
| Fraxinus | Ash | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 1% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 0% | 34% | 0% | 2% | 8% | 0% | 0% |
| Salix | Willow | 0% | 30% | 3% | 43% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 28% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 40% | 0% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 1% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 21% | 1% | 0% | 2% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 5% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 1% | 2% | 0% | 0% | 0% |
| llex | Holly | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

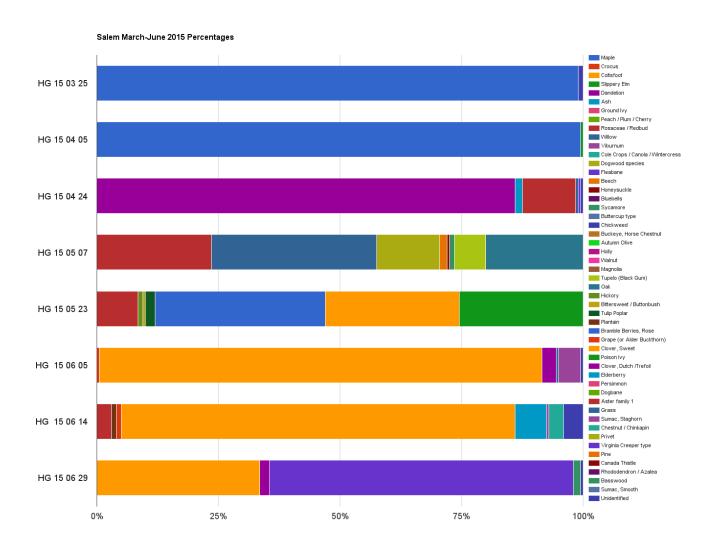
| Nyssa sylvatica Tupelo (Black Gum) 0% 0% 0% 42% 0% 0% 0% Quercus Oak 0% 0% 1% 10% 0% 0% 0% Carya Hickory 0% | Magnolia | Magnolia | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
|--|-----------------|------------------------|------|------|------|------|------|------|------|
| Carya Hickory 0% | Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 0% | 42% | 0% | 0% | 0% |
| Celastrus / Cephalanthus Bittersweet / Buttohbush 0% 0% 0% 1% 2% 0% 0% Liriodendron tulipifera Tulip Poplar 0% | Quercus | Oak | 0% | 0% | 1% | 10% | 0% | 0% | 0% |
| Cephalanthus Buttonbush 0% 0% 0% 1% 2% 0% <td>Carya</td> <td>Hickory</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> | Carya | Hickory | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| tulipifera Tulip Poplar 0% <td></td> <td></td> <td>0%</td> <td>0%</td> <td>0%</td> <td>1%</td> <td>2%</td> <td>0%</td> <td>0%</td> | | | 0% | 0% | 0% | 1% | 2% | 0% | 0% |
| Rubus / Rosa Bramble Berries, Rose 0% 0% 0% 0% 35% 0% 4% Vitis (or Rhamnus) Grape (or Alder Rhamnus) 0% | | Tulip Poplar | 0% | 0% | 0% | 0% | 3% | 0% | 0% |
| Rubus / Rosa Rose 0% 0% 0% 0% 35% 0% 4% Vitis (or Rhamnus) Grape (or Alder Bhamnus) 0% | Plantago | Plantain | 0% | 0% | 0% | 0% | 0% | 0% | 2% |
| Rhamnus) Buckthorn) 0% | Rubus / Rosa | | 0% | 0% | 0% | 0% | 35% | 0% | 4% |
| Toxicodendron radicans Poison Ivy 0% 0% 0% 0% 23% 0% 0% Trifolium Clover, Dutch; Trefoil 0% | | | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| radicans Poison Ivy 0% | Melilotus | Clover, Sweet | 0% | 0% | 0% | 0% | 29% | 87% | 88% |
| Sambucus Elderberry 0% | | Poison Ivy | 0% | 0% | 0% | 0% | 23% | 0% | 0% |
| Diospyros virginiana Persimmon 0% <t< td=""><td>Trifolium</td><td>Clover, Dutch; Trefoil</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td></t<> | Trifolium | Clover, Dutch; Trefoil | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| virginiana Persimmon 0% | Sambucus | Elderberry | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| cannabinum Dogbane 0% | | Persimmon | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Poaceae Grass 0% | | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhus typhina Sumac, Staghorn 0% | Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Castanea type Chestnut / Chinkapin 0% 0% 0% 0% 0% 0% 6% Ligustrum Privet 0% </td <td>Poaceae</td> <td>Grass</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> <td>0%</td> | Poaceae | Grass | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ligustrum Privet 0% | Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 11% | 0% |
| Parthenocussus Virginia Creeper type 0% | Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 0% | 6% |
| Pinus Pine 0% <t< td=""><td>Ligustrum</td><td>Privet</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td></t<> | Ligustrum | Privet | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense Canada Thistle 0% <t< td=""><td>Parthenocussus</td><td>Virginia Creeper type</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td><td>0%</td></t<> | Parthenocussus | Virginia Creeper type | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron Rhododendron / Azalea 0% | Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron Azalea 0% | Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhus glabra Sumac, Smooth 0% 0% 0% 0% 0% 0% Unidentified 1% 1% 1% 1% 1% 1% 1% 1% | Rhododendron | | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Unidentified 1% 1% 1% 1% 1% 1% 1% 1% | Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| 100% 100% 100% 100% 100% 100% 100% | | Unidentified | 1% | 1% | 1% | 1% | 1% | 1% | 1% |
| | | | 100% | 100% | 100% | 100% | 100% | 100% | 100% |



South of Salem WV, Harrison County. 1200 ft. elevation, about 39.263 latitude.

| Salem | 2015 | | March/Apr | il | М | ay | June | | |
|------------------------|-----------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Scientific Name | Sample #-Date ► Common Name | HG 15 03 25 | HG 15 04 05 | HG 15 04 24 | HG 15 05 07 | HG 15 05 23 | HG 15 06 05 | HG 15 06 14 | HG 15 06 29 |
| Acer | Maple | 99% | 100% | 0% | 0% | 0% | 0% | 0% | 0% |
| Crocus | Crocus | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tussilago farfara | Coltsfoot | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Ulmus rubra | Slippery Elm | 0% | 1% | 0% | 0% | 0% | 0% | 0% | 0% |
| Taraxacum officinale | Dandelion | 0% | 0% | 86% | 0% | 0% | 0% | 0% | 0% |
| Fraxinus | Ash | 0% | 0% | 2% | 0% | 0% | 0% | 0% | 0% |
| Glechoma hederaceae | Ground Ivy | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Prunus | Peach / Plum / Cherry | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rosaceae / Cercis | Rosaceae / Redbud | 0% | 0% | 11% | 24% | 9% | 0% | 3% | 0% |
| Salix | Willow | 0% | 0% | 1% | 34% | 0% | 0% | 0% | 0% |
| Viburnum | Viburnum | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Brassica | Cole Crops / Canola / Wintercress | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cornus | Dogwood species | 0% | 0% | 0% | 13% | 0% | 0% | 0% | 0% |
| Erigeron | Fleabane | 0% | 0% | 1% | 0% | 0% | 0% | 0% | 0% |
| Fagus | Beech | 0% | 0% | 0% | 2% | 0% | 0% | 0% | 0% |
| Lonicera | Honeysuckle | 0% | 0% | 0% | 1% | 0% | 0% | 0% | 0% |
| Mertensia | Bluebells | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Platanus occidentalis | Sycamore | 0% | 0% | 0% | 1% | 0% | 0% | 0% | 0% |
| Ranunculus | Buttercup type | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Stellaria | Chickweed | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Aesculus | Buckeye, Horse Chestnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Elaeagnus umbellata | Autumn Olive | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| llex | Holly | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Juglans | Walnut | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |

| Magnolia | Magnolia | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
|-----------------------------|-------------------------------|------|------|------|------|------|------|------|------|
| Nyssa sylvatica | Tupelo (Black Gum) | 0% | 0% | 0% | 7% | 0% | 0% | 0% | 0% |
| Quercus | Oak | 0% | 0% | 0% | 20% | 0% | 0% | 0% | 0% |
| Carya | Hickory | 0% | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 0% | 0% | 0% | 0% | 1% | 0% | 0% | 0% |
| Liriodendron tulipifera | Tulip Poplar | 0% | 0% | 0% | 0% | 2% | 0% | 0% | 0% |
| Plantago | Plantain | 0% | 0% | 0% | 0% | 0% | 0% | 1% | 0% |
| Rubus / Rosa | Bramble Berries, Rose | 0% | 0% | 0% | 0% | 35% | 0% | 0% | 0% |
| Vitis (or Rhamnus) | Grape (or Alder Buckthorn) | 0% | 0% | 0% | 0% | 0% | 1% | 1% | 0% |
| Melilotus | Clover, Sweet | 0% | 0% | 0% | 0% | 28% | 91% | 81% | 34% |
| Toxicodendron radicans | Poison Ivy | 0% | 0% | 0% | 0% | 26% | 0% | 0% | 0% |
| Trifolium | Clover, Dutch /Trefoil | 0% | 0% | 0% | 0% | 0% | 3% | 0% | 2% |
| Sambucus | Elderberry | 0% | 0% | 0% | 0% | 0% | 0% | 7% | 0% |
| Diospyros virginiana | Persimmon | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Apocynum cannabinum | Dogbane | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Asteraceae 1 | Aster family 1 | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Poaceae | Grass | 0% | 0% | 0% | 0% | 0% | 1% | 0% | 0% |
| Rhus typhina | Sumac, Staghorn | 0% | 0% | 0% | 0% | 0% | 5% | 1% | 0% |
| Castanea type | Chestnut / Chinkapin | 0% | 0% | 0% | 0% | 0% | 0% | 3% | 0% |
| Ligustrum | Privet | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Parthenocussus | Virginia Creeper type | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 63% |
| Pinus | Pine | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Cirsium arvense | Canada Thistle | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Rhododendron | Rhododendron / Azalea | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Tilia | Basswood | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 2% |
| Rhus glabra | Sumac, Smooth | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Unidentified | 1% | 0% | 1% | 0% | 0% | 1% | 4% | 1% |
| | | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |



Late March and Early April Comments:

Six of the seven late March and Early April samples consisted of nearly 100% Maple pollen, almost certainly Red Maple (*Acer rubrum*). This period of time represents the crucial "turnover" period as the bees' winter population must be successfully replaced by the new generation. Maple pollen is known to be a high source of protein (Crailsheim, 2013, Univ. Graz) and appears to be highly attractive to honey bees. Despite a colony's natural population being at a lower point at this time, our measurements showed that the bees gathered maple pollen in fairly large daily quantities, often surpassing a day's gathering in late June. Furthermore, honey bees well nourished at the larval stage during this maple pollen income would be expected to comprise most of the foraging force during our most productive nectar flows in May. If the pollen were to be stored as bee bread, its beneficial impact could be substantially extended. This indicates that Red Maple (*Acer rubrum*) may be one of the most valuable species for promoting honey bee health and productivity in West Virginia. Fortunately it is already an abundant species, probably universally, in our state.

The exception was the March 31 sample from southeastern Raleigh County which showed the Maple pollen at only 22% and matched by Clover pollen at 23%. The Clover pollen presented a problem, as clover could not be blooming this early in the year. The collaborator reported that many honey bees were seen on the clover hay at the feed lot adjacent to the apiary. The most abundant pollen type in this sample was a type of *Asteraceae* (also called "*Compositae*") pollen. The only plant of this type known by the project leader likely to bloom at this time would be Coltsfoot (*Tussilago farfara*) but definite distinction between many of the similar *Asteraceae* pollen types was not possible with the available resources.

Middle to late April Comments:

A couple interesting and unexpected findings in the more diversified middle to late April samples included significant amounts of Ash (*Fraxinus*) and Sycamore (*Platanus occidentalis*). The Ash pollen raises concerns regarding the spread of Emerald Ash Borer which is endangering a food source that appears to be important to pollinators in some locations.

One of the late April samples (Salem, 2015) consisted of 86% Dandelion pollen. The collaborator reported chilly and windy conditions on that day, which probably caused the bees to forage closer to the ground and closer to the hives than they would have otherwise. The amount of pollen collected per colony on that date was very small and no other sample showed even half that percentage of Dandelion.

One of the biggest issues faced during the whole project was the similarity of Redbud (*Cercis Canadensis*) pollen to some of the *Rosaceae* pollen types such as Hawthorn, Pear, Apple, Serviceberry, and their ornamental varieties. We decided to lump all these together under the title "Rosaceae / Cercis" in the reports. This was an important issue to us because these pollen grains represented a very significant percentage of some of the samples. Some of the *Rosaceae* pollen types are known to provide good protein and nutrition levels for colony growth, while the protein content of Redbud pollen is unknown. Differentiation between them may become possible at a later date. All the prepared pollen and slides will be kept for future reference.

The variety and types of pollen acquired by the bees in the month of April led us to believe that the bees were not lacking sufficient nutrition.

May Comments:

It was interesting to see the differences between locations in May. In Raleigh County at 3,000 ft. elevation, we continued to find significant amounts of Ash (*Fraxinus*) and "*Rosaceae*". In late May an abundance of a spiny *Asteraceae* type emerged that was rare in all the other locations.

Amounts of Poison Ivy (*Toxicodendron radicans*) pollen varied from 10% to over 80%, but never showed up in any the Raleigh County samples. It should be noted that Tree-of-Heaven (*Ailanthus*) pollen grains are very similar to those of Poison Ivy, but the grains found in the samples resembled Poison Ivy better, and other references indicated that Tree-of-Heaven bloom occurred in June rather than May.

Sweet Clover (Melilotus) pollen showed up in late May universally.

One common theme among all the Harrison County May samples was Willow pollen, evidently Black Willow, which blooms later than Pussy Willow. We expected to see Blackberry type pollen common in all the samples, but it only showed up in the Salem and Quiet Dell samples.

June Comments:

The most universally important species showing up in June had to be Sweet Clover (*Melilotus*) followed by Chestnut (*Castanea*). The chestnut type could be wild-growing Chinkapin trees (*Castanopis*) or introduced Chinese, European, or Japanese cultivars which are commonly planted by homeowners. At the Salem location, the amount of White Dutch Clover (*Trifolium repens*) in the vicinity far exceeded the amount of Sweet Clover (*Melilotus*), yet the Sweet Clover pollen always comprised a significant amount while the White Dutch Clover did not.

Occasionally other types would comprise a significant percentage of the June samples such as Elderberry (Sambucus) or Virginia Creeper (Parthenocissus). By the pollen grains in the samples we could not distinguish between Virginia Creeper (P. quinquefolia), False Virginia creeper (P. vitacea), and Boston Ivy (P. tricuspidata), but the collaborators reported a lot of Virginia Creeper in their areas and were unaware of any Boston Ivy.

It was common to see the weight of harvested pollen drop off significantly in late June. The reason for this is unknown, but it would be interesting to see what kind of impact could be had if a greater amount of Sweet Clover or Chestnut forage was made available to the bees.

A mystery emerged with the June 28 sample from Jackson County. The sample contained 96% Maple (Acer) pollen with a scattering of a few other April pollen types. It was a very small pollen harvest of only 0.00625 lb (1/10 oz.) from four traps. The strangest part was that there was no trace of any pollen from the season in which it was trapped. The project leader checked the unprepared pellets from the original vial submitted by the collaborator, which agreed with the analysis. The collaborator said each trap had a little and it did not all come from one trap. One explanation involved some excess pollen from previous trappings which had been fed on wax paper inside another hive about 1-1/4 miles away but it did not

seem likely that bees from all four colonies would have actually entered that hive and robbed the pollen. There were no other known beekeepers in the area.

2013-2015 Year Comparisons at the Salem location:

A unique feature of the Salem samples was the Black Tupelo ("Black Gum") (Nyssa sylvatica) pollen which turned out to be an important early-mid May type in 2013 and 2014 but not so much in 2015. Another surprise was the large amount of Dogwod (Cornus) pollen in the 2014 Salem sample. The collaborator had previously looked for honey bees on the abundant flowering dogwoods in the area and concluded that they were used by bees very little or not at all. After planting a Silky Dogwood (C. amomum) he noticed bees very actively gathering pollen from the blooms, but would not have expected the bees to derive 40% of their pollen from that one tree, seeing that it was not a scanty harvest and there were many hives at the location.

The April 18 2014 sample matched the April 26 2013 sample much better than the April 18, 2013 sample. It appears the bloom dates were pushed later in 2013 or earlier in 2014 due to factors such as the weather. However by late May the 2013 and 2014 samples were extremely similar.

The large amount of Virginia Creeper (*Parthenocissus*) pollen in the 2015 sample was completely absent in the 2013 and 2014 samples. Two large Virginia Creeper vines in two apple trees near the hives had always been cut back, but had been allowed to grow in 2014 and bloom in 2015. The collaborator noticed and photographed honey bees gathering nectar but not pollen from the flower clusters of these two vines on July 13, but did not notice any late June activity. There were at least two dozen colonies in the apiary so even though the harvest on June 20 was small, it seems surprising that so many colonies would have obtained nearly two thirds of their pollen from those two vines.

Differences between sites:

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 tend to support the former, that when a variety of plants are in bloom, there are significant differences in foraging even between two



fairly close locations. Reasons for this are speculative, likely due to very 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 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.

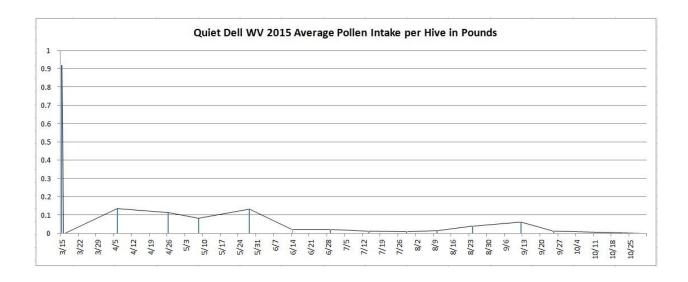
Average Pollen Intake per Hive in Pounds

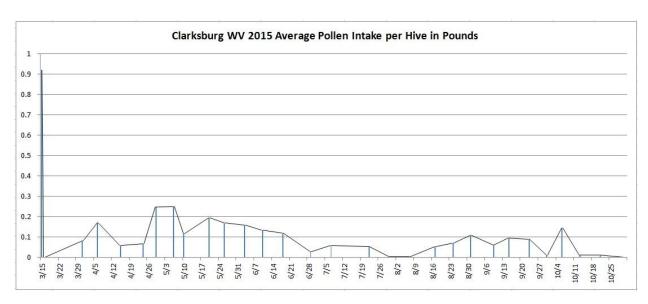
Below is a series of graphs showing the amount of pollen brought in through the year by weight. It is important to remember that the pollen was collected on favorable foraging days, which can be scarce at times due to unfavorable weather. The true average pollen intake therefore may be lower than the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

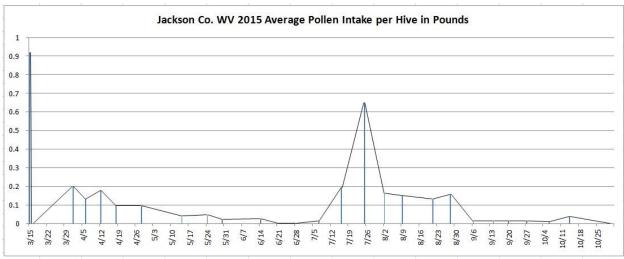
The vertical axis in the graphs is weight in pounds. The highest mark is one pound, about the maximum that could be collected in one day in our area with this method of collection. Continuous trapping would stimulate the bees to compensate by collecting much larger amounts. Collecting in short single-day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

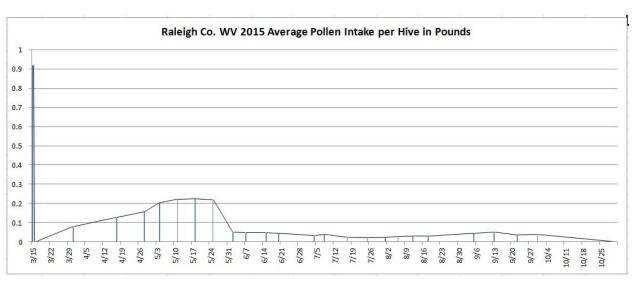
The dates at the bottom show 1-week intervals from March to October, while the vertical lines show points at which samples were taken. The height of the vertical lines shows the amount of pollen collected in that sample, corresponding to the weights on the vertical axis. (Ignore the high vertical line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

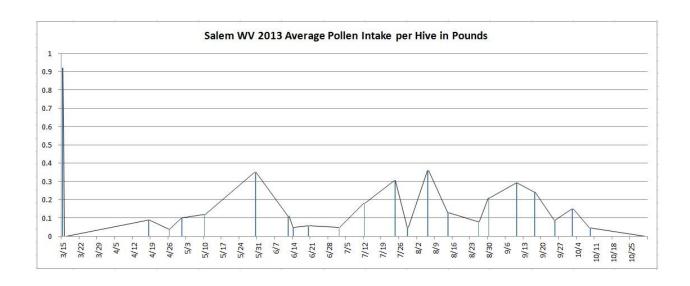
If more information was known about the protein content of each pollen type, a similar chart could be compiled showing the amount of protein being gathered by the bees at each point through the active season.

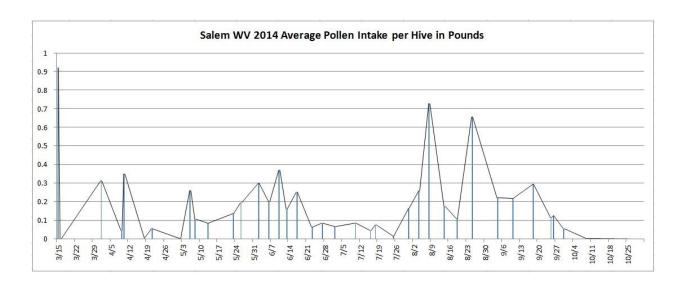


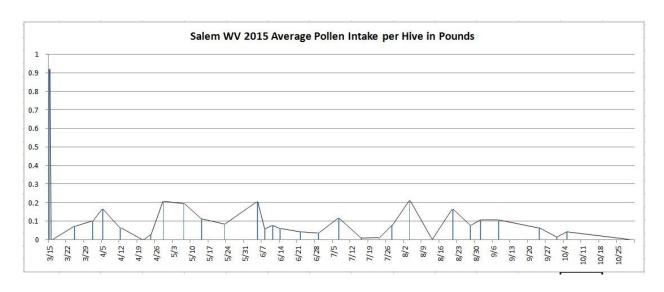












Pollen Importance Tables, March-June

Pollen Type Importance to Honey Bees, by % of Total Daily Intake (TDI) and weight Organized by maximum percentage found in any sample March through June WV Pollen Project 2015

| Scientific Name | Common Name | Max % of TDI | Max weight /hive /day, lbs | * max % and wt not same sample |
|--------------------------|--------------------------|--------------|----------------------------|--------------------------------|
| Acer | Maple | 100.00% | 0.31094 | * |
| Melilotus | Clover, Sweet | 91.00% | 0.31919 | * |
| Rubus / Rosa | Bramble Berries, Rose | 88.00% | 0.14350 | * |
| Rosaceae / Cercis | Rosaceae / Redbud | 86.00% | 0.09856 | |
| Taraxacum officinale | Dandelion | 86.00% | 0.02183 | |
| Parthenocissus | Virginia Creeper type | 62.50% | 0.02227 | |
| Castanea type | Chestnut / Chinquapin | 50.50% | 0.01263 | |
| Toxicodendron radicans | Poison Ivy | 49.50% | 0.06497 | |
| Nyssa sylvatica | Tupelo ("Black Gum") | 46.00% | 0.05520 | |
| Platanus occidentalis | Sycamore | 44.50% | 0.11032 | |
| Prunus | Peach/Plum/Cherry | 42.50% | 0.03825 | |
| Salix | Willow | 41.00% | 0.06658 | * |
| Cornus | Dogwood species | 40.00% | 0.10400 | |
| Asteraceae 1 | Aster family 1 | 39.50% | 0.08639 | |
| Fraxinus | Ash | 35.00% | 0.04441 | |
| Tussilago farfara | Coltsfoot | 33.50% | 0.02652 | |
| Viburnum | Viburnum | 27.50% | 0.07150 | |
| Quercus | Oak | 27.00% | 0.03917 | * |
| Lonicera | Honeysuckle | 20.50% | 0.05330 | |
| Plantago | Plantain | 19.00% | 0.00903 | |
| Diospyros virginiana | Persimmon | 15.50% | 0.00339 | |
| Ranunculus | Buttercup type | 12.50% | 0.00719 | |
| Rhus typhina | Sumac, Staghorn | 11.00% | 0.04059 | |
| Sambucus | Elderberry | 10.50% | 0.01155 | |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 10.00% | 0.01200 | |
| Trifolium | Clover, Dutch; Trefoil | 10.00% | 0.02187 | |
| Carya | Hickory | 8.50% | 0.01020 | |

| Fagus | Beech | 8.00% | 0.01983 |
|-------------------------|--------------------------------------|-------|-----------|
| Erigeron | Fleabane | 7.00% | 0.00817 |
| Ligustrum | Privet | 6.00% | 0.00345 |
| Vitis | Grape | 5.00% | 0.00844 |
| Aesculus | Buckeye, Horse Chestnut | 3.00% | 0.00250 |
| Liriodendron tulipifera | Tulip Poplar | 3.00% | 0.00578 |
| Mertensia | Bluebells | 3.00% | 0.00173 |
| Tilia | Basswood | 3.00% | 0.00173 |
| llex | Holly | 2.50% | 0.00875 |
| Elaeagnus umbellata | Autumn Olive | 2.00% | 0.00219 * |
| Juglans | Walnut | 2.00% | 0.00240 |
| Stellaria | Chickweed | 2.00% | 0.00191 |
| Magnolia | Magnolia | 1.50% | 0.00033 |
| Brassica | Cole Crops / Canola / Wintercress | 1.00% | 0.00095 |
| Cirsium arvense | Canada Thistle | 1.00% | 0.00021 |
| Glechoma hederaceae | Ground Ivy | 1.00% | 0.00260 |
| Poaceae | Grass | 1.00% | 0.00110 |
| Apocynum cannabinum | Dogbane | 0.50% | 0.00010 |
| Pinus | Pine | 0.50% | 0.00067 |
| Rhododendron | Rhododendron / Azalea | 0.50% | 0.00023 |
| Rhus glabra | Sumac, Smooth | 0.50% | 0.00015 |
| Ulmus rubra | Slippery Elm | 0.50% | 0.00083 |
| Crocus | Crocus | 0.00% | 0.00000 |

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 March through June, WV Pollen Project 2015

| Scientific Name | Common Name | Max % of TDI | Max weight /hive /day, lbs | * max % and wt not same sample |
|--------------------------|--------------------------|--------------|----------------------------|--------------------------------|
| Melilotus | Clover, Sweet | 91.00% | 0.31919 | * |
| Acer | Maple | 100.00% | 0.31094 | * |
| Rubus / Rosa | Bramble Berries, Rose | 88.00% | 0.14350 | * |
| Platanus occidentalis | Sycamore | 44.50% | 0.11032 | |
| Cornus | Dogwood species | 40.00% | 0.10400 | |
| Rosaceae / Cercis | Rosaceae / Redbud | 86.00% | 0.09856 | |
| Asteraceae 1 | Aster family 1 | 39.50% | 0.08639 | |
| Viburnum | Viburnum | 27.50% | 0.07150 | |
| Salix | Willow | 41.00% | 0.06658 | * |
| Toxicodendron radicans | Poison Ivy | 49.50% | 0.06497 | |
| Nyssa sylvatica | Tupelo ("Black Gum") | 46.00% | 0.05520 | |
| Lonicera | Honeysuckle | 20.50% | 0.05330 | |
| Fraxinus | Ash | 35.00% | 0.04441 | |
| Rhus typhina | Sumac, Staghorn | 11.00% | 0.04059 | |
| Quercus | Oak | 27.00% | 0.03917 | * |
| Prunus | Peach/Plum/Cherry | 42.50% | 0.03825 | |
| Tussilago farfara | Coltsfoot | 33.50% | 0.02652 | |
| Parthenocissus | Virginia Creeper type | 62.50% | 0.02227 | |
| Trifolium | Clover, Dutch; Trefoil | 10.00% | 0.02187 | |
| Taraxacum officinale | Dandelion | 86.00% | 0.02183 | |
| Fagus | Beech | 8.00% | 0.01983 | |
| Castanea type | Chestnut / Chinkapin | 50.50% | 0.01263 | |
| Celastrus / Cephalanthus | Bittersweet / Buttonbush | 10.00% | 0.01200 | |
| Sambucus | Elderberry | 10.50% | 0.01155 | |
| Carya | Hickory | 8.50% | 0.01020 | |
| Plantago | Plantain | 19.00% | 0.00903 | |
| llex | Holly | 2.50% | 0.00875 | |
| Vitis | Grape | 5.00% | 0.00844 | |

| Erigeron | Fleabane | 7.00% | 0.00817 |
|-------------------------|--------------------------------------|--------|-----------|
| Ranunculus | Buttercup type | 12.50% | 0.00719 |
| Liriodendron tulipifera | Tulip Poplar | 3.00% | 0.00578 |
| Mertensia | Bluebells | 3.00% | 0.00173 |
| Ligustrum | Privet | 6.00% | 0.00345 |
| Diospyros virginiana | Persimmon | 15.50% | 0.00339 |
| Glechoma hederaceae | Ground Ivy | 1.00% | 0.00260 |
| Aesculus | Buckeye, Horse Chestnut | 3.00% | 0.00250 |
| Juglans | Walnut | 2.00% | 0.00240 |
| Elaeagnus umbellata | Autumn Olive | 2.00% | 0.00219 * |
| Stellaria | Chickweed | 2.00% | 0.00191 |
| Tilia | Basswood | 3.00% | 0.00173 |
| Poaceae | Grass | 1.00% | 0.00110 |
| Brassica | Cole Crops / Canola / Wintercress | 1.00% | 0.00095 |
| Ulmus fulva | Slippery Elm | 0.50% | 0.00083 |
| Pinus | Pine | 0.50% | 0.00067 |
| Magnolia | Magnolia | 1.50% | 0.00033 |
| Rhododendron | Rhododendron / Azalea | 0.50% | 0.00023 |
| Cirsium arvense | Canada Thistle | 1.00% | 0.00021 |
| Rhus rubra | Sumac, Smooth | 0.50% | 0.00015 |
| Apocynum cannabinum | Dogbane | 0.50% | 0.00010 |
| Crocus | Crocus | 0.00% | 0.00000 |

Comparison to North Carolina Study:

A study was conducted by Louisiana State University in 2013 in which 61 pollen pellet samples were collected at 12 apiaries in north-central North Carolina at 6 collection times and analyzed for the pollen types present. A list of the predominant (>43%), secondary (16-42%), important minor (3-15%) and minor (<3%) pollen types was produced. As promised in the WV Pollen Project Proposal, the results of that study are compared with the results of the WV Pollen Project in a table which lists the pollen types ranked by importance in North Carolina (all types) adjacent to the pollen types ranked by importance in West Virginia (March through June only). The exact collection dates for the North Carolina study were not divulged in the report so it is not possible to make an equal comparison. By the pollen types shown, it appears the NC collection times did not begin as early as our study, and continued into the late summer / fall season.

| North Carolina Comparison Table | | | | | | | | |
|---------------------------------|-----------------------|------------------|------------------------|-----------------------|--|--|--|--|
| Scientific Name | Common Name | * late season | Scientific Name | Common Name | | | | |
| N | orth Carolina | | Wes | st Virginia | | | | |
| Predominant (>43%) | | | | | | | | |
| Parthenocissus | Virginia Creeper type | | Acer | Maple | | | | |
| Melilotus | Clover, Sweet | | Melilotus | Clover, Sweet | | | | |
| Plantago | Plantain | | Rubus / Rosa | Bramble Berries, Rose | | | | |
| Rhus [copallinum?] | Sumac [Winged?] | * | Rosaceae / Cercis | Rosaceae / Redbud | | | | |
| Asteraceae-Senecio type | Goldenrod type | * | Taraxacum officinale | Dandelion | | | | |
| | | | Parthenocussus | Virginia Creeper type | | | | |
| | | | Castanea type | Chestnut / Chinkapin | | | | |
| | | | Toxicodendron radicans | Poison Ivy | | | | |
| | | | Nyssa sylvatica | Tupelo (Black Gum) | | | | |
| | | | Platanus occidentalis | Sycamore | | | | |
| | Sec | ondary (| (16-42%) | | | | | |
| Magnolia | Magnolia | | Prunus | Peach/Plum/Cherry | | | | |
| Trifolium | Clover / Trefoil | | Salix | Willow | | | | |
| Poaceae | Grass | | Cornus | Dogwood species | | | | |
| Ranunculaceae | Buttercup family | | Asteraceae 1 | Aster family 1 | | | | |
| Fagopyrum esculentum | Buckwheat | | Fraxinus | Ash | | | | |
| Gleditsia | Locust | | Tussilago farfara | Coltsfoot | | | | |
| Unidentified 3-colp. | | | Viburnum | Viburnum | | | | |

| Apiaceae | Carrot family | | Quercus | Oak |
|---|--------------------------------------|----------|-----------------------------|--------------------------------------|
| Lagerstroemia indica | Crape Myrtle | | Lonicera | Honeysuckle |
| Amaranthaceae /Chenopodiaceae | Amaranth/Goosefoot families | | Plantago | Plantain |
| | | | Diospyros virginiana | Persimmon |
| | Importan | t minor | types (3-15%) | |
| Asteraceae (Lactuceae or Cichorieae type) | Dandelion type | | Ranunculus | Buttercup type |
| Asteraceae (Heliantheae type) | Ragweet type | | Rhus typhina | Sumac, Staghorn |
| Primula? | Primrose type | | Sambucus | Elderberry |
| Asteraceae (Heliantheae type) | Sunflower type | | Trifolium | Clover, Dutch; Trefoil |
| Zea mays | Corn (Maize) | | Celastrus / Cephalanthus | Bittersweet / Buttonbush |
| Diodia teres | Poorjoe | | Carya | Hickory |
| Cirsium | Plume thistles | | Fagus | Beech |
| Brassicaceae | Cole Crops / Canola / Wintercress | | Erigeron | Fleabane |
| | | | Ligustrum | Privet |
| | | | Vitis (or Rhamnus) | Grape (or Alder Buckthorn) |
| | | | Liriodendron tulipifera | Tulip Poplar |
| | | | Mertensia | Bluebells |
| | | | Aesculus | Buckeye, Horse Chestnut |
| | | | Tilia | Basswood |
| | Mi | nor type | s (<3%) | |
| Vitis | Grape | | llex | Holly |
| Leucophyllum | Barometerbush | | Juglans | Walnut |
| Tsuga | Hemlock (conifer) | | Elaeagnus umbellata | Autumn Olive |
| Eucalyptus | Eucalyptus | | Stellaria | Chickweed |
| Convolvulus-like | Bindweed-like | | Magnolia | Magnolia |
| Ludwigia | Water-primrose | | Glechoma hederaceae | Ground Ivy |
| Fern Spore | Fern | | Poaceae | Grass |
| Portulaca | Purslane | | Brassica | Cole Crops / Canola / Wintercress |
| Polygonum | Knotweed, Smartweed | | Cirsium arvense | Canada Thistle |

| Oxydendrum arboreum | Sourwood | | Ulmus rubra | Slippery Elm |
|---------------------|---|---|---------------------|-----------------------|
| Lilium? | Lily? | | Pinus | Pine |
| Camellia? | Camellia? | | Rhododendron | Rhododendron / Azalea |
| Euphorbiaceae? | Spurge family | | Rhus glabra | Sumac, Smooth |
| Glycine max | Soybean | * | Apocynum cannabinum | Dogbane |
| Ulmus zelkhova | Zelkhova (Ulmaceae fam., genus Zelkhova.) | * | | |
| Lonicera | Honeysuckle | * | | |
| Oenothera | Evening primrose | * | | |
| Impatiens | Jewelweed, Touch-me- not | * | | |
| Ambrosia | A ragweed | | | |

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 many 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 is both reduced brood production and reduced vitality of individual bees, then it is easy to see how the nutrition and mite issues compound one another as skyrocketing varroa infestation rates spiral a colony into decline.

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. late June) and indicates the types of plants that could be used to increase the available forage at that time. Continuation of the study through the rest of the summer will add to this knowledge.

Two plants that are eagerly utilized by bees for pollen and could be used to improve the quantity of late June forage are Sweet Clover (*Melilotus*) and Chestnut (*Castanea*). Chestnut pollen is about 23.5% protein, however the document showing Chestnut pollen's protein level was not adjusted for what the bees are able to obtain due to its specific amino acid profile. If the ratio is not correctly balanced, the

bees may not be able to use the full 23.5% protein from Chestnut pollen. Other possibilities for increasing late June bee forage are Virginia Creeper (*Parthenocussus*) and Elderberry (*Sambucus*). The nutritional quality of these is unknown to us at this point.

Planting Chestnut trees or patches of sweet clover could be money well spent by the beekeeper. There are indications that as few as one or two trees can provide a substantial amount of pollen for a dozen colonies. There are also other potentially beneficial aspects to these particular plants beyond their usefulness to honey bees.

Conclusions and Contributions

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

The study revealed a common occurrence of low pollen intake in June, the awareness of which can help beekeepers understand colony dynamics and improve colony management.

As a result of the project, one of the collaborators determined to change the location of his hives.

At the West Virginia Beekeepers Association Fall Conference the main speaker, Tom Seeley of Cornell, twice expressed his excitement about our research into the pollen types coming into a hive, calling it "an amazing investigation". Such a compliment from an individual of such stature was unexpected and very encouraging.

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.

Results from the March and April analysis was put into a power point and delivered at the West Virginia Beekeepers Association fall conference at Jackson's Mill on September 25, 2015. The project design and methods were explained along with interesting results-to-date.

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.

A final outreach report including the tables and bar graphs was compiled into a PDF document and submitted to the West Virginia Beekeepers Association web master on December 29 for posting on the

WVBA website. It was also posted on the Honey Glen Blog and e-mailed to beekeepers and interested individuals statewide.

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. There is a continued need for more complete reference materials. Although references exist for some species in most of the common genera, there were numerous species common in our region for which we did not have any reference photographs. March- to June-blooming species for which reference photos are needed include Black Haw Viburnum (V. prunifolium), Sugar Maple (Acer saccharum), Silver Maple (A. saccharinum), Wintercress (Barbarea vulgaris), Oriental Bittersweet (Celastrus orbiculatus), Northern Red Oak (Quercus rubra), White Oak (Q. alba), Black Oak (Q. velutina), Chestnut Oak (Q. prinus), Silky Dogwood (Cornus amomum), and Staghorn Sumac (Rhus typhina). Better quality images or a size reference are needed for many additional species.

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, size of grains, bloom time of the species, and pointing out the differences between similar species would be ideal.

The project leader wishes to express his sincere appreciation to Mike Blessing, Harold Davis, Steve Hamrick, Mark Lilly, Professor Vaughn Bryant, Dr. Don Trisel, Assistant Professor Zach Fowler, Dr. Larry Campbell, and the whole Staddon family for enthusiastically offering their services, expertise, and cooperation in support of this project.

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.