

Protocol for National Honey Bee Pest and Disease Survey

Project Plan

Details of the Project Plan for 2018 are available at

http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/SurveyProjectPlan.pdf

Please read this protocol carefully and watch the training video

https://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/National%20Bee%20Survey%20Video_small.wmv prior to initiating sampling. For additional information, email faheybrl@umd.edu.

List of equipment

For all apiaries (Fig 1)

- ✓ 1 wash tub
- ✓ 1 metal pan
- ✓ ¼ cup measuring cup
- ✓ 2 funnels
- ✓ 1 gallon bottle (to be filled with water)
- ✓ 1 squeeze bottle (to be filled with water)
- ✓ 1 gallon of chlorine bleach (to be used for cleaning equipment)
- ✓ beekeeping equipment (not provided) – smoker, lighter, bee veil, etc.
- ✓ writing pen
- ✓ 3 large Priority Mail flat rate boxes (to mail alcohol samples)
- ✓ 3 pre-addressed mailing labels
- ✓ 1 inch wide transparent shipping tape
- ✓ strainer
- ✓ 1 bucket
- ✓ binder clips
- ✓ 1 pair of scissors

- ✓ Informative brochures for participating beekeepers

Per apiary (contained in live bee shipping box; Fig 2A)

- ✓ 1 live bee shipping box
- ✓ 1 pre-addressed mailing label
- ✓ 1 set of 4 stickers with identical identification numbers
- ✓ 1 data sheet
- ✓ 1 large (500 mL) bottle with alcohol
- ✓ 1 small (125 mL) bottle with alcohol
- ✓ 1 cloth filter
- ✓ queen candy in petri dish on bottom of the live bee shipping box
- ✓ 1 capped 15 mL tube filled with water and a sponge
- ✓ *Tropilaelaps* Information Sheet
- ✓ 1 pre sampling survey and return envelope with postage

Overview: The goal is to collect a composite sample of adult bees from 8 randomly selected colonies in each apiary that you sample. You will need to open 8 colonies and remove a frame that contains young developing brood and shake the adult bees into the collection wash tub. You will collect two, ¼ cup scoops of bees. One scoop of bees will go into alcohol bottle and the second scoop of bees will be placed in the live bee box for that apiary. You will also “bump” a single brood frame from each colony to dislodge exotic parasitic mites like *Tropilaelaps* and/or pests such as the small hive beetle. You will leave the apiary with a composite sample of live bees in the ventilated cardboard box and a composite sample of bees in alcohol in the large alcohol vial. You will also have the filtered wash from the comb “bump” in the smaller alcohol bottle. For specifics on how to collect the samples see steps below.

Steps:

1. Before leaving for apiary ensure all equipment is on hand including beekeeping protective gear and beekeeping tools. **IMPORTANT:** remember to fill the one gallon bottle and squeeze bottle with water.
2. Identify apiary with at least 10 colonies of bees (8 of which will be sampled, with 2 extra in case inspector encounters dead outs or queen-less colonies during inspection. Dead outs and queen-less colonies should not be included in the survey sampling). Prioritize queen producers, package/nuc producers, honey producers, and apiaries used for crop pollination. Select apiaries in areas at high risk for exotics invasion (near deep water shipping ports, international airports, and high traffic areas for migratory beekeeping). Apiaries should be chosen in order to give as close to an equal representation of the entire state as possible. Ideally, a state will be sectioned into 4 quadrants with apiaries randomly chosen from each quadrant.
REMINDER: Split up your sampling trips so you are not sampling all of your beekeepers within the same season. Take samples in the spring, summer and fall.
3. Also identify the nearest post office to this apiary from which you can mail the live sample of bees immediately after sample collection or if mailing several boxes the boxes must be mailed by the end of the day before the Post Office closes.
 - a. It is best to ship live bees between Monday and Wednesday so they do not arrive at the The UMD Honey Bee Lab on the weekend when there is no one available to receive them. Please do not sample and ship live bees on Thursday.
 - b. It is legal to mail live bees. Go to https://pe.usps.com/text/pub52/pub52c5_008.htm for more information on the postal service regulations.
4. Explain the sampling process to the beekeeper. Show the beekeeper the *Tropilaelaps* mite information sheet and answer any questions they may have. Have the beekeeper fill out the pre-sampling survey and mail it the UMD lab.
5. Open one live bee mailing box (Fig 2B), it should contain:
 - a. Data sheet and ID stickers.
 - b. Large bottle and small bottle of alcohol.
 - c. Petri dish glued to box floor containing queen candy.
 - d. Capped 15 mL tube filled with water and a wicking sponge.
 - e. Cloth filter.

- f. Mailing label.
 - g. Data identification sheet.
6. Place identifying stickers onto (Fig 3):
 - a. Data identification sheet.
 - b. Large collection bottle containing alcohol.
 - c. Small collection bottle containing alcohol.
 - d. Live bee shipping box.
 7. Fill out the data information sheet. An electronic version of the datasheet that can be filled out on the computer and emailed to faheybrl@umd.edu. It is available at http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/apiary-dis.pdf.
 8. Set up sampling equipment:
 - a. Remove wax paper covering queen candy (in petri dish and glued to floor of box) (Fig 4).
 - b. Remove cap from 15 mL tube containing the wet sponge to allow bees access to water source. Place tube in live bee box. (Fig 5).
 - c. Close shipping box making sure the two sides with metal screen match up to ensure proper ventilation for live bees and place funnel into hole into top of box (Fig 6A).
 - d. BE SURE TO KEEP THE LIVE SHIPPING BOX IN THE SHADE AND OUT OF DIRECT SUNLIGHT AND WIND.
 - e. Open large alcohol bottle and place second funnel into its mouth so it can receive bees (Fig 6B). It may be helpful to place the alcohol bottle in the center of a roll of duct tape to stabilize the bottle.
 9. When sampling an apiary, it is critical to select colonies at random, which is different than haphazard or regularly spaced. Colonies should under no circumstances be preferentially selected because they seem “healthy” or “sickly”. To help select colonies as random, use the attached pages of randomly generated numbers (pages 16 and 17).
 10. Open colony to the brood nest and examine for disease and queen status/condition. Record any disease/queen conditions on data sheet.
 11. Find a frame containing at least some uncapped brood (Fig 7). Ensure queen is not present on this frame. IF no brood is present, and another colony is available, do not sample the broodless colony but choose another colony with brood. IF no other colony is available take adult bee sample from a frame in the center of brood nest. If you have a choice, try and chose a well wired, sturdy frame to sample.
 12. Shake bees from frame into wash tub (Fig 8).
 13. Knock the wash tub to gather bees into one corner of the tub (Fig 9).
 14. Scoop ¼ cup of adult bees (Fig 10) into the funnel inserted into the lid of the live bee shipping box (Fig 11). Gently tap the box to force the bees from the funnel into the box (Fig 12). Do not collect more than ¼ cup of bees because overcrowding will result in mortality during shipping.
 - a. BE SURE TO KEEP THE LIVE SHIPPING BOX IN THE SHADE AND OUT OF DIRECT SUNLIGHT AND WIND.

- b. It also may be helpful to put the lid from the 500 mL alcohol bottle over the opening of the funnel to reduce the risk of live bees escaping. This may also help you keep track of the lid.
15. Scoop a second $\frac{1}{4}$ cup of bees from the tub into the funnel inserted in the large bottle containing alcohol, tap the bottle and funnel to force bees into the bottle (Fig 13).
16. Take the frame of bees (from which the bees were removed in step 9), and hold the frame over the metal collection pan, with one surface of the frame facing down (Fig 14). Firmly knock the outer edge of the frame in the pan possibly dislodging mites, beetles and other hive debris from the frame into the collection pan (Fig 15).
17. Knock the frame a second time.
18. Flip the frame so the frame surface facing down in step 15 is now facing up.
19. Knock the frame twice in the same manner outlined in step 14.
20. Switch the frame so you are now holding the opposite end of the frame and repeat steps 14-17. Each frame should be flipped 2 times, so that the frame is knocked a total of 8 times (2 knocks, flip over, 2 knocks, switch end of frame you are holding, 2 knocks, flip over, 2 knocks).
21. Place the brood frame back into the colony, and dump any bees remaining in the collection wash tub back in the colony.
22. Close the colony.
23. Repeat steps 8 through 22 until 8 colonies have been sampled. Note we are sampling at the apiary level, so samples from all colonies are being placed in the same live shipping box, large alcohol collection box, and debris from 8 frames from 8 different colonies are being knocked into the same metal tray. You will basically be collecting two scoops of live bees from each of 8 hives and placing them in either the live bee shipping box or the alcohol bottle and also “knocking” a single brood frame from each hive over the large metal tray.
24. Close the large bottle containing alcohol and tip the bottle several times dampening the contained bees (Fig 16).
25. Attach the cloth filter into the strainer using the binder clips (Fig 17).
26. Place about 2 cups of water into the metal collection pan and gently swirl the contents (Fig 18) before pouring the water and debris through the cloth filter (Fig 19). Be sure not to let water flow over the edge of the cloth filter.
27. Repeat step 24 until most of the debris in the collection pan has been removed, then using the squeeze bottle remove what debris remains and pass this through the cloth filter (Fig 20).
28. Allow the cloth filter to drip dry for a couple of minutes, and then gently remove it from the strainer, folding it so that the filtered debris remains secure in the center of the filter (Fig 21). This debris will be examined for mites, specifically *Tropilaelaps*.
29. Place the entire gently folded cloth filter into the small bottle containing alcohol and seal the bottle tightly to avoid spills during transport (Fig 22). Please make sure all of the filter material is in the main part of the bottle and none is on the neck of the bottle. Save the binder clips to be reused at subsequent apiaries.
30. Double check the lids of the small and large collections to make sure they are tight. Place both the small (containing cloth filter) and large bottle (containing bees) into large ziplock bags to contain any leaks from the alcohol before placing them into the large flat rate shipping box. This

box should be mailed when the box is full. Prior to mailing, place one of the pre-addressed shipping labels (UMD Honey Bee Lab, College Park, MD) at the appropriate place on the large Priority Mail flat rate box. Write FROM and your return address on the upper left corner of the box.

31. Ensure the data collection sheet is completely filled out and legible. Please make a copy of this sheet for your records, and then place it into a ziplock bag (with any other data sheets you have already filled out). The ziplock bag containing the data sheets should be sent with the mailing box containing sample bottles (Fig 23).
32. Remove the funnel from the live bee shipping container and cover the hole with the cardboard flap (Fig 24).
33. Secure the hole and lid of the live bee shipping box to the bottom of the box with the transparent shipping tape (Fig 25).
34. Place the mailing label (UMD Honey Bee Lab, College Park, MD) over the now sealed hole on the top of live bee shipping box and secure it with tape (Fig 26). Write FROM and your return address on the upper left corner of the box. Write the date on the front of the box. Write "Surface Mail Only" on box. Please ensure that the sample label is clearly visible on the live bee box and is not covered by tape or postage.
35. **Drop off the live bee shipping box, containing collected samples, at the nearest post office ASAP. Honeybees can only be shipped via surface transportation (USPS Ground).**
36. Collect and clean sampling equipment (pans, hive tools, funnels, and ¼ cup scoop) with 10% bleach and water solution. You may also sanitize hive tools by scorching them in your smoker.
37. Email: National Honey Bee Pest and Disease Survey Coordinator Rachel Fahey (faheybrl@umd.edu) within 24 hours of shipment to notify the UMD lab that a live bee shipment is expected. Please also email Rachel Fahey within 24 hours after shipping alcohol and filtered samples.



Figure 1: Reusable equipment needed for sampling apiaries. Materials include (a) 1 gallon bottle (to be filled with water), (b) wash tub, (c) strainer, (d) metal pan, (e) funnel, (f) clear binding tape, (g) squeeze bottle (to be filled with water), (h) binder clips, (i) scissors, (j) training video, (k) ¼ cup measuring cup, (l) bucket, (m) 3 large Priority Mail flat rate boxes, (n) letters explaining the purpose of this sampling (a copy of this should be given to cooperating beekeepers), (o) pen, (p) 3 preaddressed mailing labels, (q) *Tropilaelaps* information sheet.

Also needed are basic beekeeping protective equipment (coveralls, veil, etc.) and beekeeping tools (smoker, hive tool etc.) which are not provided.



Figure 2A: A live bee shipping box which contains (Figure2B) all the materials needed to collect and store and ship samples in one apiary.

Figure 2B: Contents of a live shipping box (a) main box and (b) box lid, (c) Petri dish glued to shipping box floor containing queen candy, (d) capped 15 mL tube filled with water and a sponge, (e) 1 small (125 mL) bottle with alcohol (for *Tropilaelaps* sample), (f) 1 large (500 mL) bottle with alcohol (for *Nosema/Varroa* sample), (g) data sheet, (h) mailing label, (j) set of 4 stickers with identical Identification numbers, and (k) cloth filter.



Figure 3: Place identification sticker onto (a) data sheet, (b) large bottle, (c) small bottle, and (d) mailing box.



Figure 4



Figure 5



Figure 4: To ensure queen candy does not dry out before use, it has been sealed in the petri dish. Remove wax paper before placing bees into the live shipping box.

Figure 5: Bees require water during transport, so the test tube with the sponge and water should be **UNCAPPED** before placing bees into the live shipping box.

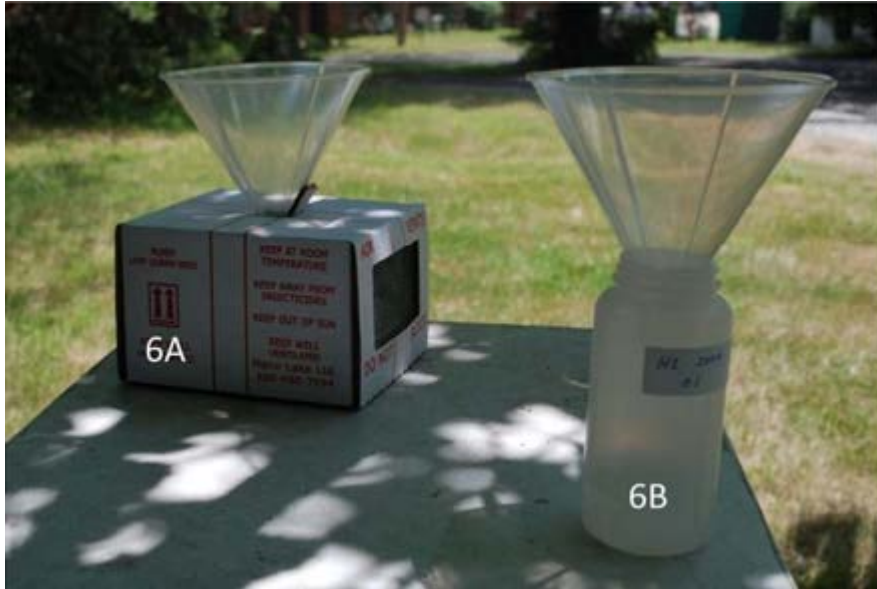


Figure 6A: Live bee shipping box with funnel inserted to receive bees. **This box should be set up and KEPT IN THE SHADE AT ALL TIMES after bees have been introduced, otherwise the sampled bees may overheat and die. Keep this box out of the wind.**

Figure 6B: Large bottle with funnel ready to receive bee samples.



Figure 7: A frame containing sufficient adult bees, uncapped and capped brood for sampling.

Figure 8



Figure 9

Figure 8: Removing bees from frame into wash tub.

Figure 9: Knock bees into corner of tub to facilitate easy removal of $\frac{1}{4}$ cup samples of live adult bees.



Figure 10: A brimming $\frac{1}{4}$ cup of bees.



Figure 11



Figure 12

Figure 11: Place bees from cup into funnel inserted in the live bee shipping box.

Figure 12: Gentle tap live bee shipping box and funnel so bees are forced into the collection box.



Figure 13: Place a second $\frac{1}{4}$ cup of bees into large bottle of alcohol.



Figure 14: Hold brood frame (from which bees have been removed) above metal collection pan, with one frame surface facing down. Firmly knock the frame over the metal collection pan by allowing one end bar to hit the metal collection pan.

Figure 15: Debris dislodged from frames after being knocked in collection pan.



Figure 16: Close the large sampling bottle containing 2 cups of bees (1/4 cup from 8 different colonies), tightly seal the lid of the container, and tip the bottle several times to ensure all bees are damp with alcohol.



Figure 17



Figure 19

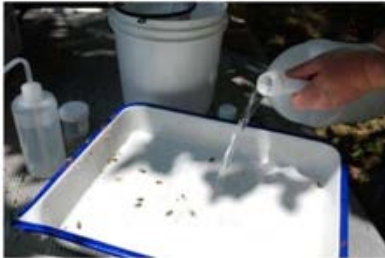


Figure 18

Figure 17: Place cloth filter over strainer.

Figure 18: Place water into metal collection pan to dislodge debris.

Figure 19: Pour water and debris from metal collection pan through the cloth filter.



Figure 20



Figure 21



Figure 22

Figure 20: Using squeeze bottle, ensure last of debris in metal collection pan is filtered through cloth filter.

Figure 21: After allowing filter to drip dry for a minute, gently fold filter so debris is secure.

Figure 22: Place folded filter into small bottle with alcohol. Secure the bottles lid.



Figure 23: After ensuring the data collection sheet has been filled out completely, and after making a copy of this sheet for your records, place it in a ziplock bag and place this ziplock in the prepaid mailing box containing the sample bottles. When ready to ship samples, add packing material (e.g., newspaper), if necessary, so bottles are not rolling around in the box. Add sealed ziplock bag with data sheets and seal box with binding tape. Attach pre-addressed label. Clearly write FROM and your return address. Email UMD Survey Coordinator Rachel Fahey (faheybrl@umd.edu) within 24 hours of shipment to notify UMD that a box of samples is expected.



Figure 24



Figure 26



Figure 25

Figure 24: Secure the hole in the lid of the live bee shipping box with clear binding tape.

Figure 25: Secure the lid of the live bee shipping box to the bottom of the box using the clear binding tape that is provided.

Figure 26: Place pre-addressed shipping label (UMD Honey Bee Lab, College Park, MD) on box. Clearly print "FROM" and your return address on the top left corner of box. Write "Surface Mail Only" on box. Make sure ID label is legible and securely attached to box. **Drop off the live bee shipping box, containing collected live bees, at the nearest post office ASAP. Honeybees can only be shipped via surface transportation (USPS Ground). See #35 above for additional information.**

Random number sheet

Instructions on how to use this sheet to select colonies in an apiary for sampling:

- ① determine the size of the apiary (n colonies)
- ② pick the first unique 8 numbers $\leq n$ to appear in the list
- ③ those are the colonies you will sample (order of sampling doesn't matter)
- ④ in next apiary, continue from where you stopped in this list; at the end of the page, loop back to the beginning

Note:

- If any of the colonies selected is unsuitable to be sampled (queenless or deadout), replace the colony with another identified as the next number ($\leq n$) from the list.
- The random number sheet covers integers between 1 and 100. In the eventuality the apiary has more than 100 colonies, flip a coin to identify a quadrant (containing less than 100 colonies) that you will focus on in this sampling event.

Example:

We are about to sample an apiary containing 40 colonies. We assign a temporary number to each colony, the number "1" being the uttermost colony in the apiary (located in a corner or at the end of a line, depending on the configuration of the apiary). The idea should be that colonies are assigned their number before you know which will be selected.

The 8 first unique occurrences of numbers ≤ 40 in the list are: 18, 35, 9, 12, 31, 27, 24 and 34.

Sample the 9th, 12th, 18th, 24th, 27th, 31st, 34th and 35th colonies of the apiary. The 24th colony in the apiary is unfortunately queenless, so we skip it and proceed to colony 26th (the next integer ≤ 40 to appear in the list).

Why is this process important?

This selection method ensures that the colonies sampled are truly selected at random. Humans are terrible at picking things at random! We tend to associate random with equally distributed. We also tend to be unconsciously attracted by things that catch our attention: an active hive entrance, buzzing, a colony with (more or less) number of supers... Only a random selection process ensures that the sample is truly representative of the population of interest. That means the person sampling colonies should have the least impact on the selection. In case of doubt, flip a coin!

Random number sheet

88	98	77	41	67	90	18	3	86	59	16	93	17
74	87	38	44	26	45	63	77	91	57	8	35	84
57	66	95	48	18	44	51	36	54	65	59	85	14
81	1	18	18	4	66	78	38	37	100	92	79	87
18	22	80	10	27	30	34	68	4	25	65	88	36
65	95	90	30	99	62	82	32	67	2	3	75	57
35	53	86	3	85	69	97	82	32	30	52	84	18
9	82	7	21	65	54	99	49	81	9	5	55	9
96	68	99	4	18	68	42	1	92	13	14	62	17
12	22	19	99	38	61	49	78	22	81	14	88	39
50	86	45	100	31	37	57	26	37	17	34	11	5
59	85	54	90	17	58	86	10	2	93	56	93	53
31	64	72	46	49	82	85	33	67	74	35	54	92
49	55	61	21	22	66	92	8	29	59	70	41	52
78	74	87	77	46	17	12	69	80	20	26	64	28
27	57	52	4	38	27	88	78	95	67	91	61	49
27	1	100	63	41	25	83	1	18	43	15	61	96
97	75	19	85	74	83	4	8	81	89	13	47	5
49	85	58	93	38	87	40	36	1	58	91	10	52
51	80	42	47	87	69	68	98	78	79	45	2	35
91	43	93	62	55	48	93	78	43	8	66	43	19
80	12	51	59	90	21	86	55	28	52	22	12	72
69	54	15	4	72	15	73	85	19	98	10	54	86
49	23	63	94	24	57	83	88	73	81	9	50	64
35	27	41	1	46	99	31	34	30	22	27	32	79
24	54	58	69	42	69	11	84	6	3	39	88	11
85	33	99	49	80	77	55	76	88	94	32	6	96
81	50	53	51	81	74	32	58	91	7	41	57	49
34	87	4	81	52	16	91	80	86	34	97	7	46
84	90	73	36	42	100	22	22	48	26	83	98	22
41	50	21	23	42	52	68	56	89	36	55	55	51
26	38	27	14	49	46	16	16	15	57	66	60	19
89	1	33	34	50	55	86	10	5	29	52	4	61
4	94	81	68	53	15	12	17	8	83	50	91	47
7	96	79	9	43	15	71	83	16	65	6	68	99
56	70	40	52	68	14	4	52	77	87	78	67	25
26	99	87	32	3	74	56	8	89	29	99	42	30
79	19	69	27	66	24	64	92	77	50	79	90	83
8	53	63	46	2	76	18	91	13	59	72	82	2
8	21	83	97	47	7	96	93	19	22	59	94	95
88	73	99	10	8	24	39	18	5	26	2	26	85
6	46	70	45	10	53	72	97	93	74	92	63	81
100	79	100	4	96	14	4	49	76	14	41	11	53
93	12	84	75	20	4	35	6	92	28	62	47	16
10	42	22	3	71	57	11	67	18	13	32	85	2
63	48	42	34	42	31	80	29	47	11	26	76	21
5	81	47	76	2	44	69	70	26	35	88	91	57
80	78	4	25	96	95	21	38	26	53	83	49	47
59	38	45	33	64	42	89	13	14	56	88	35	17
58	48	43	57	25	48	28	45	53	95	13	41	58
26	29	66	85	74	16	74	79	46	100	84	97	66
36	51	13	45	30	63	66	39	74	96	88	68	90
2	72	48	68	83	39	87	16	38	42	79	63	39
74	27	22	39	71	69	40	51	11	75	29	82	48
55	21	88	45	85	93	48	47	48	100	48	22	84
77	37	39	21	41	76	21	48	46	30	94	26	64
21	43	49	6	78	3	17	81	77	31	67	86	16
73	33	18	79	92	29	75	21	43	22	91	81	3