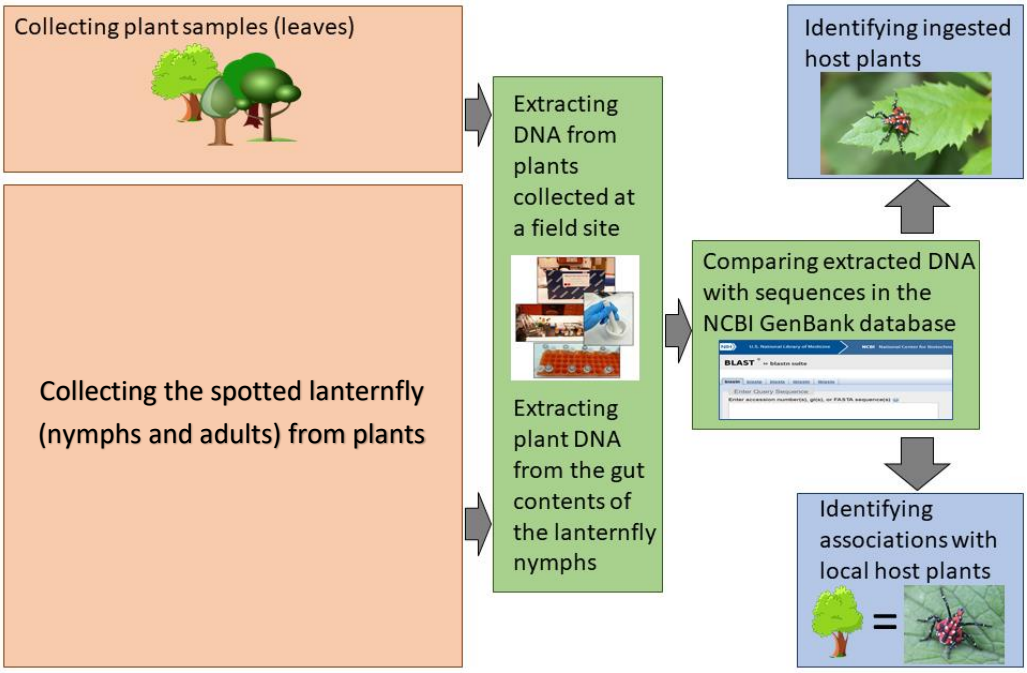
Protocol for the spotted lanternfly and host plant collection

Overall goal for insect and plant collection:



Part 1. Field site: things to record:

- Date of collection
- Air temperature
- Time of collection
- Sunny/cloudy/rainy, etc,
- Field site area (approximately, ideally >= 10×10 m^2)
- Plant composition: %cover of woody/non-woody plants, # of different woody plants if possible, tree height, min and max distance between trees
- Lanternfly stages present at the field site: adults (with wings), 4th instar (red with black dots), 3rd instar (almost as big as 4th but black with white dots), 2nd instar (small black with white dots), 1st instar (even smaller than 2nd one, black with white dots, it looks like a tick; present in May-June only)

Part 2. Collecting plant samples for DNA work:

Equipment to have in the field: marker, Ziplock bags, disposable rubber gloves, cooler, ice packs

Protocol:

1. Put on the gloves.
2. Clip 1-2 leaves from each plant present at the field site.
3. Put leaves in a separate Ziplock bag (1 bag per plant).
4. Label the bag as "Field site # /Plant #" (for example, "FS1/PL1")
5. Put the bag in a cooler with ice (dry-ice is the best, but regular one is fine too)

Part 3. Collecting the lanternfly for DNA work (ideally it should be done together with collecting the plant samples to match the labels):

Equipment to have in the field: marker, Ziplock bags or microcentrifuge tubes, disposable rubber gloves, cooler, ice packs

Protocol:

1. Put on the gloves.
2. Grab an individual nymph (or adult) from a plant.
3. Put the nymph in a separate Ziplock bag or a microcentrifuge tube (ideally).
4. Label the bag/tube as "Field site # /Plant #/Lanternfly stage #/Individual letter or number" (for example, for two 3rd instar nymphs collected from the same plant the labels would be: "FS1/PL1/4a" and "FS1/PL1/4b"; for two adults, a male and female the labels would be: "FS1/PL1/M1" and "FS1/PL1/F1")
5. Put the bag/tube in a cooler with ice (dry-ice is the best, but regular one is fine too)
6. Collect ~30 lanternfly individuals per stage and/or ~5-10 individuals of each stage from each plant (whichever is possible)
7. After collection, transport the cooler with both plant and lanternfly samples to the lab (or home) and put all the bags in the freezer (-20^{0}C).

Part 4. Alternative plant collection protocol (without subsequent DNA work):

<u>For morphological plant ID in the lab:</u>

Equipment to have in the field: marker, 'post-it'-notes, paper sheets, folder or press, phone

Protocol:

1. Prepare a bunch of thin paper sheets (newspaper is the best) and 'post-it'-notes
2. Take a picture of the plant you want to ID.
3. Clip the top of the plant (~20cm) with leaves and flowers if possible
4. Put the plant between paper sheets and press the pile.
5. Attach the label (as for DNA work); add a note about plant height, plant life form, and image #.
6. Transfer the paper sheets with plants to the lab. No freezing is needed.

<u>For morphological plant ID in the field:</u>

Equipment to have in the field: marker, notebook, phone

Protocol:

1. Download any plant identification app on your phone:
   - I used PlantNet Plant Identification in the past: https://play.google.com/store/apps/details?id=org.plantnet&hl=en_US It's very easy and pretty reliable.
   - I haven't used these ones yet, but they seem to be commonly used, too: PlantSnap: https://play.google.com/store/apps/details?id=org.plantnet&hl=en_US PictureThis: https://play.google.com/store/apps/details?id=cn.danatech.xingseus&hl=en_US
2. Take a picture of the plant you want to ID.

3. Use this picture to ID the plant using any plant identification app, but also save the image.
4. In a separate notebook, record plant ID, image#, and a label (same way as for DNA work).

Part 5. Additional notes:

- Plant and lanternfly samples can be stored in the freezer at -20^{0}C 'forever'.
- When using a piece of plant sample for DNA extraction make sure to proceed with DNA extraction immediately and store the rest of the leaf in the freezer right away (frozen plant tissues decay quickly at room temperature)
- For DNA extraction from the lanternfly gut contents, please use a whole body of 1st, 2nd, and 3rd instar nymphs. 4th instar: please remove legs and use the body only. Adults: please remove legs and wings and use half of the body for DNA extraction (you might need to have 2 samples with 2 body halves and then combine the sequencing results)