Collecting Nectar and Pollen Samples:  
*Methods and Cautions*

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Knowledge of residues in pollen and nectar resulting from applications of crop protection products can facilitate assessment of potential risk to pollinators…

provided the data reflect the true post-application conditions.
Our field study experience has demonstrated various pitfalls that can result in spurious results and contaminated control samples

Areas of Concern

Application timing Vs. Plant Physiology
Protecting Test Plots from Pollinators
Nectar and Pollen Extraction Procedures
Anther Dehiscence: *The process of anthers opening to expose and/or expel pollen*

Anthers in many types of flowers have specific dehiscence temperatures or temperature range, below or above which the anthers do not open.
Anthers not open - pollen not visible
Dehisced Anthers - Almond Flower
Dehisced Anthers and Stripped Anthers - Orange Flower
Some examples of dehiscing temperatures:

Almonds in CA – 57 degrees F
Bing Cherry in OR – 45- 48 degrees F
Cotton in LA - 82 degrees F (day time)

can vary by crop variety
The timing of foliar pesticide application, relative to anther dehiscence, can be the difference between minimal chemical deposition on pollen and a worse-case deposition scenario.

A worse case scenario may be the objective in some regulatory residue studies. But frequently, a minimal deposition scenario is the objective.
Foliar application before dehiscence with pollen collection after dehiscence is the minimum residue scenario.
Pollinators are Often a Source of Undesirable Contamination of Pollen on Residue Study Plots
Protecting the Study Plot

To assure clean control samples, and unbiased residue determinations, plants on the study plot should be protected to prevent pollinator access.

Net-covered hoops are necessary for some crops.
When analyzing at the PPB level, extreme caution must be applied when collecting pollen and nectar samples.
Flowers should not be picked by hand if it can be avoided.
With certain crops, picked flowers should not touch one another.

The same flowers should not be used to collect both nectar and pollen.
Cotton Flower - Late Candle Stage
Cut-away Cotton Flower
When vacuuming pollen, tiny pieces of anther and filament can be sucked into the collection pipette tip. The fragments may have higher residue levels than the pollen.
Multiple filters in vacuum devices, each with different pore diameter, help separate unwanted fragments from pollen.
Nectar is often drawn from the flower cup using capillary tubes, which can inadvertently touch pollen particles and transfer them into the nectar.

Each capillary tube should be used in just a single flower to reduce likelihood of transferring pollen into nectar.
Contaminated nectar sample

Capillary tubes used to draw nectar can introduce pollen granules into the nectar.

At PPB level of detection, this can represent a measurable increase in chemical concentration of the sample.
In some cases, pollen can be removed from a flower without actually touching or probing into the flower. Methods such as this can reduce likelihood of sample contamination.

Cranberry flower and device for vibrating the flower at the frequency of bumble bee wing beat.
Bees can collect samples more efficiently than humans with less contamination…in certain plants

Vs.
In some plants, no pollen or no nectar sample will be available. Bees have little interest in pollen from some plants, but still collect the nectar.
Some plants or varieties do not produce nectar or pollen, or have less favorable physiology or chronology for continuous bee collection. Some plants require bumble bees or other non-apis bees for collection.

- **Tomatoes** (No nectar)
- **Peppers** (No nectar)
- **Oranges** (Pollen-variety)
- **Soybeans** (No pollen)
- **Sunflowers** (No pollen)
- **Strawberries** (Not suitable for hand collection)
- **Alfalfa** (No pollen)
• Considerations for tree crops:
  • Age
    • Crop destruct
    • Blossom production
  • Size
  • Variability of bloom times between varieties
  • Attractiveness to bees (if applicable)
Issues with bees in a tunnel:

- Longevity of the hive
- Size of the hive
- Weeds in the tunnel
- Aggression
Low exposure levels of concern for pollinators and highly sensitive analytical capability combine to emphasize the need for exceptionally cautious sample collection. Key considerations include:

• Application timing relative to anther dehiscing and clear statement of objectives from the study sponsor
• Protection of flowers to be sampled from pollinator access to avoid contamination of pollen – especially in control plots
• Sampling techniques that guard against nectar sample contamination
• Thorough consideration of appropriate sampling methodology for the crop under consideration