**Breh’s Watermelon Project Protocol**

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**Seeding and Planting of watermelon**

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   Description automatically generatedSeeds will be started in 72 cell trays with ProMix B with Mycorrhizae potting soil (e.g., Wooster Madison Greenhouse 112). Seeds will be started on April 24-25, 2024.
   1. Seedless watermelon cultivar” ‘Liberty’
      1. Fungicide treated – Farmore F300
   2. Pollenizer watermelon cultivar: ‘SP6’ (Syngenta)
      1. 1-2 lb fruit with yellow flesh
2. Plants will be hardened off in outdoor greenhouse structure (Wooster OARDC greenhouse)
   1. Half of transplants moved on 5/16, other half of transplants moved on 5/20
3. For bed preparation, cultivated & tilled fields had rows of black plastic mulch and drip tape installed. Rows are 5-6 ft. apart, and spray alleys were incorporated based on sprayer capabilities.

**Figure 1:** General field layout with the pollenizers (SP6) in the perimeters and the recipient plant (Liberty) inside this perimeter.

1. Transplants will be planted in 25-36 rows across the field with approximately 73 plants per row.
   1. Row #s and plants per row may change slightly based on the location’s field sizes
   2. Plants will be separated by 3 ft. within rows
   3. Pollenizer plants will be planted as the entire perimeter of the field (see Fig. 1)
2. Following transplant, fields will be scouted 1-2 times weekly to determine need for any replant & to monitor for cucumber beetle pressure

**Field Locations**

1. Treatments will be replicated at 2 sites, Wooster, OH, and South Charleston, OH
   1. Within each site, fields are separated by at least 3000 ft (1 km)
   2. At each site, during each visit, the sequence of treatments sampled will switch to avoid sampling bias based on the time of day.
2. Field Setup & Maintenance:
   1. Pollenizers will be planted in a single row around the perimeter of each field, and the inner area will be planted with the recipient (Liberty) plant. See Fig 1.
   2. Insecticide applications will be planned per the specific treatment (see Treatment section below & Table 2).
      1. Some of the field locations in Wooster may potentially face corn rootworm pressure, so these will be closely scouted for potential infestations
         1. Western Corn Rootworm (WCR) can become a problem in cucurbits later in the season once nearby corn has matured. They do not feed on cucurbit roots, but instead can cause damage to the blossoms and fruit ([Hoffmann & Zitter 1994](https://ecommons.cornell.edu/server/api/core/bitstreams/b622eacc-a784-471b-8971-f545c706de3c/content)).
         2. Female adult WCR looks very similar to SCB. However, the differences between the two are shown in Table 1.

**Table 1. SCB and WCR ID**

|  |  |
| --- | --- |
| Striped Cucumber Beetle (SCB) | Western Corn Rootworm (WCR) |
| striped cucumber beetle -Acalymma vittatum | Western Corn Rootworm | USU |
| Cucumber Beetles | Wisconsin Vegetable Entomology | Corn Rootworm Prevention and Control | Gardener's Supply |
| * Black abdomen * Defined stripes | * Yellow abdomen * Blurry stripes |

* + 1. The first 2-3 weeks following transplants, the plants are particularly vulnerable to striped cucumber beetle pressure. The fields will be closely scouted during this period to determine if insecticide applications are needed.
       1. 15 plants will be randomly selected per field (edges & within), and the number of SCB will be recorded to determine the average SCB per plant. In the initial 3 weeks post-transplant, if SCB/plant is 2 beetles per plant or higher, an insecticide application will be made.
       2. After the first 3 weeks, fields will continue to be scouted weekly, using the same method described above, but with an action threshold of 5 striped cucumber beetles per plant to warrant an insecticide application.
          1. When plants begin to grow together, instead of counting the beetles per plant, the beetles on the plant surface in a 1 m2 quadrat will be used instead.
       3. For SCB scouting, check the entire plant surface, especially under the foliage.
    2. Presence of other pests will be recorded and monitored to determine if other insecticide/miticide applications are needed.
  1. Fungicide applications will be made using [MELCAST](https://melcast.ceris.purdue.edu/) & scouting on both treatments
  2. Following bed-making, herbicides Strategy and Sandea will be applied around the plot & between rows for preemptive weed control.

1. Treatments:
   1. Two treatments, conventional & IPPM/untreated, managed as seen below in Table 2:

**Table 2. Treatment Insecticide Application Schedule**

|  |  |  |
| --- | --- | --- |
|  | **Conventional** | **IPPM/Untreated** |
| **At Transplant** | Drench w/ Imidacloprid (Admire Pro @ 10 fl. Oz. per acre) – *6/1/24 (Wooster)/ 6/4-5/24 (Western)* | - |
| **Week 1** | - | \* |
| **Week 2** | - | \* |
| **Week 3** |  | \**Assail 30SG application @ 5.3 oz per acre + Surfactant – 6/18/24 (Wooster)* |
| **Week 4** | Foliar application of Belay @ 4 fl. Oz. per acre – *6/27/24 (Wooster)* | \* |
| **Week 5** | *(Potential) Foliar application (Endigo)/(Potential) Foliar application (Pounce)* | - |
| **Week 6** |  | - |
| **Week 7** |  | - |
| **Week 8** | Foliar application (Pounce) | - |
| **Week 9** | Foliar application (Pounce) | - |
| **Week 10** | Foliar application (Pounce) | - |

\*Assail foliar applications as needed for IPPM/Untreated treatment

**Pollinator Data Collection**

1. **A screenshot of a computer

   Description automatically generatedField Zones**: To understand pollination as the distance gets further from the pollenizer plants, each field will be split into four “zones” (See Fig 2). These zones will be flagged for easy ID. Below the field zones and other features are described further:
   1. ***Pollenizers*:** The entire perimeter of the field will be planted with a single row of pollenizer plants.
   2. ***Zone 1*:** This area is marked in the dark green on the field map. It designates the space 0-10 ft. from the pollenizers. This area has a very high likelihood of successful pollination and fruit set.
   3. ***Zone 2:*** This area is marked in the light green on the field map. It designated the space 10-30 ft. from the pollenizers. This area has a medium likelihood of successful pollination and fruit set.
   4. ***Zone 3:*** This area is marked in the light blue on the field map. It designates the space 30-60 ft. from the pollenizers. This area has a low likelihood of successful pollination and fruit set.

**Figure 2:** Zone map for field. The yellow perimeter is the pollenizer plants. The black columns are spray alleys, and the remaining-colored blocks are different zones of the field.

* 1. ***Zone 4:*** This area is marked in dark blue on the field map. It designates the space 70 ft. to the remainder of the field (dependent on the size of the field). This area has the lowest likelihood of successful pollination & fruit set.

1. **Pollinator Observations** (beginning at primary bloom, mid-July to early August, 3-4 weeks, 6-8 separate observation sets for each field site)
   1. On a day of pollinator observations, one location will be chosen (Wooster or Western), and both fields will have pollinator observations completed within that day and the 8-12 AM pollinator activity window. The observations will also be planned around low wind speeds (<16 km/h), no rain, and little cloud cover.
   2. Within each zone for each plot, 4 sampling locations will be selected at random (one on each side of the field). Each sampling location will cover a 1m2 area of watermelon plants. For each sampling location, pollinators will be observed for 4 minutes. Specific details recorded will be…
      1. Number of pollinators
      2. Type of pollinators (see ID sheets in Google Drive)
      3. Duration of visits
      4. Estimated number of male and female flowers within sampling location (see Figure 3 for ID)

A hand holding a plant

Description automatically generated

**Female flower** (bulbous growth, small melon attached)

**Figure 3.** Male and Female Watermelon Flower ID**.** Female flowers will have a bulbous growth below petals, while male flowers will not.

**Male flower**

(no bulbous growth)

* 1. ***Specifics for Conventional fields*:** Each week, one set of pollinator observations will occur at most 24 hours after the weekly insecticide application. Observations will need to be timed with insecticide applications to ensure that during the 24-hour post-application period, we will be able to get to the site for the 8-12 AM observation window. This means that no spray applications can occur on the same day at both locations. Depending on the REI of the insecticide and time post-spray, Tyvek suits & latex gloves may need to be worn. If possible, another set of pollinator observations will be conducted the morning before a spray application or the day before the application. This will allow for data to be collected before and directly after sprays.
  2. ***Specifics for IPPM/Untreated fields*:** Pollinator observations of the IPPM/Untreated fields will be paired with the conventional observations. If observations are occurring due to a spray application in conventional fields, observations will also occur at the IPPM/Untreated field on the same day at that location. Because the pollinator window is short, it will be super important to collect all data needed in a timely manner.

1. **Pesticide Residue Testing** (beginning at primary bloom)
   1. Following pollinator observations, pollinators will be collected via a bug vacuum from the field. Using the vacuum up to 20 bees will be collected per field, in a period of 30 minutes. These will be returned in vials placed in labeled ziploc bags. Aim to collect larger pollinators (Bumblebees, honeybees, squash bees, long-horned bees), as these are more likely to have larger pollen deposits than smaller bees (sweat bees).
      1. These will be returned to the lab in an insulated cooler and frozen for later pesticide residue testing. The residue testing will be looking for the presence of neonicotinoids & pyrethroids. Protocols for sample preparation and residual testing will be described in more detail later in the field season.
         1. Residue testing will consist of testing pollen present on collected pollinators
            1. Done via Precision Glycerine Jelly Swab technique described [here](https://besjournals.onlinelibrary.wiley.com/doi/pdf/10.1111/2041-210X.13863)
         2. As well as testing the pollinators themselves
            1. Done via modified QuEChERS method to extract pesticides from samples, described [here](https://www.sciencedirect.com/science/article/pii/S0048969715306331?casa_token=b3Poir1nCC0AAAAA:mN9oBrLhBBKdmCXeYnLqmKps_tutox43cEpagaDVHryc7ov0kASilVS9SwhQ38mNscQfW4OEiCY#bb0085)
      2. Following lab prep of samples, these will be sent to the Bindley Bioscience Center at Purdue University for analysis
   2. Along with collections of pollinators after sprays and before, pollinators will also be collected 2 days post-spray at various times during the season. This will be used to see the potential degradation in residues that may occur between directly-before and after sprays.

**Non-Pollinator Data Collection**

1. **Flower Collection for Pesticide Residue Testing & Pollen Deposition**:
   1. After an insecticide application is made, 2 male flowers (See Fig. 3 for ID Info) from each sampling site (= 8 flowers from each zone) will be collected and placed into individual, labeled tubes.
   2. Tubes will be returned to the lab in a cooler and stored for future use.
   3. Collected flowers will be frozen and stored until sample processing for:
      1. Pesticide residue testing, as described for pollinator pesticide residue testing (samples sent to Bindley Bioscience Center at Purdue University)
         1. The flowers for residue testing can be stored upon return to the lab at 4ºC for up to 48 hours.
         2. The stigma will be removed from the flowers using sterilized forceps. The stigma & any deposited pollen grains will be weighed up to 3 g, put in a centrifuge tube, and homogenized with a sterile pestle.
         3. The homogenized samples are then stored at -80ºC until residue analysis.
2. **Pollen deposition analysis & Pollination Event Observation** (following pollinator observation period)
   1. This will be conducted after primary bloom/pollinator observations to prevent any impact on yield. Although timed sprays are not occurring in the IPPM field, the bagging will be mimicked in those fields to compare pollen deposition and successful pollination events across treatments.
   2. Insecticide applications will need to be timed (especially in Western) to ensure that rebagging is possible at the designated time post-exposure.
   3. 24 hours before an insecticide application, 12 unopened female flowers from each zone will be bagged with mesh paint strainer bags. At the time they are bagged, they will also be labeled depending on their fate post-insecticide application. Affix the label close to the female fruit that is bagged.
      1. 4 of the selected flowers in a zone will be marked with red tape at bagging, 4 will be marked with blue tape at bagging, and 4 will be marked with orange tape at bagging (see Figure \_\_)
   4. Directly before an insecticide application, mesh bags will be gently removed from all selected female flowers for insecticide exposure. The flowers will then be gently rebagged at various time points depending on the colored label.
      1. Flowers marked with red tape will be rebagged 2 hours after insecticide exposure (~10 AM).
      2. Flowers marked with blue tape will be rebagged at 6 hours after insecticide exposure (~4 PM)
      3. Flowers marked with orange tape will be rebagged 24 hours after insecticide exposure (~8 AM the following day)
   5. Upon rebagging, 2 of the 4 flowers for each exposure time in each zone will be collected in tubes and returned to the lab. These will be destructively sampled to determine pollen deposition.
      1. These flowers will be stored in the lab at room temperature for 24 hours to allow pollen to adhere to the stigma. Following this period, the stigma will be removed, softened with 10% KOH, and stained with fuchsin.
      2. Stigma specimens will be observed through microscope slides and the number of pollen grains present will be counted using a compound microscope.
   6. The remaining 2 flowers for each exposure time in each zone will remain in the field to determine successful pollination events. The mesh bags will be gently removed in the afternoon of the day following insecticide application. The labels will remain affixed near the female fruit.
      1. One week following the removal of the bags, the flowers that remained in the field will be observed for successful fruit set or abortion to determine whether successful pollination occurred.

**Watermelon yield and assessment**

Watermelon yield and quality may differ based on pollination visitation and beetle damage. The USDA’s ag and markets specifies that watermelon grades correlate to watermelon quality. Specifically, seedless watermelons should be free of external damage, hollow heart, and have no more than 10 hard seeds per watermelon. Once watermelon begin to ripen (tendril closes to the fruit will turn brown), we will continuously harvest melons from plots and destructively sample each melon to determine the yield and quality. Confused on when to harvest? Check out this YouTube clip for more detail: <https://www.youtube.com/watch?v=EDX3yL2bnu4> or this one: <https://www.youtube.com/watch?v=2kjOnnhcLfs> (start video at 0.56 seconds).



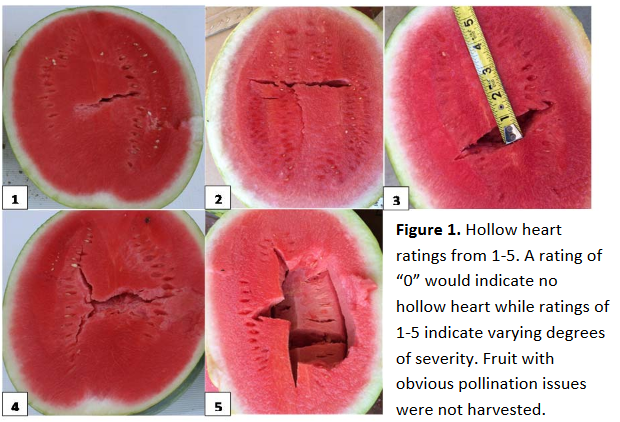
**Figure 4:** Exterior of watermelon heavily damaged by cucumber beetle feeding. You are unlikely to see this severe of damage, but its likely you will see some amount of external feeding.

1. **Locate & label melons with QR codes when ready for harvest:** Starting in the outer zone and working our way in, one person will oversee tagging melons that are ready for harvest with a QR code sticker. At the time of marking, these melons will be scanned using an ArcGIS application (still troubleshooting this). This application will tag the GPS coordinates within the field and associate the QR code with that location & melon.
2. **Weigh every melon:** Once the melon has been tagged and location-marked, the melon can be removed from the field and weighed. The QR code on the melon will associate it with the data collected for that melon that is recorded on the datasheet.
3. **Inspect every melon externally and internally. Metrics described below:**
   1. Inspect melons for exterior damage (e.g. beetle feeding damage, figure 4).
      1. Present? Y/N
      2. Scale? 0-4 (see below Figure for calibration)

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| Watermelon PNG Transparent Clipart​ | Gallery Yopriceville - High-Quality  Free Images and Transparent PNG Clipart | Watermelon PNG Transparent Clipart​ | Gallery Yopriceville - High-Quality  Free Images and Transparent PNG Clipart | Watermelon PNG Transparent Clipart​ | Gallery Yopriceville - High-Quality  Free Images and Transparent PNG Clipart | Watermelon PNG Transparent Clipart​ | Gallery Yopriceville - High-Quality  Free Images and Transparent PNG Clipart | Watermelon PNG Transparent Clipart​ | Gallery Yopriceville - High-Quality  Free Images and Transparent PNG Clipart |
| **0**  No beetle feeding (0%) | **1**  1-2 small areas of beetle feeding  (<5%) | **2**  3-5 small areas of beetle feeding or 1-2 large areas  (5-15%) | **3**  >15%, but <50% rind impacted by beetle feeding | **4**  <50% of rind impacted by beetle feeding |

**Figure 5.** Beetle rind damage rating for use during harvest.

* 1. Cut melon longitudinally and assess heart tissue. Determine hollow heart severity rating as described in Figure 6.



**Figure 6:** Shown from left to right are hollow heart severity ratings. 0= no hollow heart, no crack in the middle whatsoever. 1-5 represent varying degrees of hollow heart, where the internal crack in the heart tissue widens with increasing hollow heart severity.

* 1. Count the number of hard seeds in the watermelon (dark brown seeds, rather than white seeds which are characteristic of seedless watermelons).
  2. Record weight, external defects, hollow heart severity rating, and the total number of seeds in the watermelon in the datasheet.