SCREENING FOR DOWNY MILDEW RESISTANCE IN CUCURBITS

Background

This screening can be done either in the screenhouse or in the field. The screening will reflect cucurbits grown under field conditions whilst being able to control the variables often associated with natural field inoculation conditions. It is essential that all plants be in a similar condition – age, height, vigour etc. The causal agent, *Pseudoperonospora cubensis*, infects all cucurbits. Individual races of *P. cubensis* are not known.

Field Screening

Weather conditions should be suitable for inoculation – moist humid conditions preferably with night-time temperatures above 15° and below 25°C which allow for the formation of a film of water on the leaves.

Simple leaf stapling method

A. Inoculation cucurbit lines with *Pseudoperonospora cubensis*

1. First thing in the morning on the day of the inoculation, check downy mildew infected source material to ensure that material is available for inoculation at 16:00.
2. Determine how many lines need to be inoculated. At least 5 plants from each line need to be inoculated in addition to the positive (???) and negative (Big-C) controls. Only one leaf per plant needs to be inoculated.
3. At 15:30, collect infected leaves. Choose the leaves with large amounts of fungal growth on the underside of the leaves. Maintain them in plastic bags in ice boxes with ice.
4. In the lab, confirm presence of the fungi using the microscope. This is only necessary for one leaf.
5. Proceed to the field. Adhere the infected leaves to “middle-aged” leaves of the test and control lines using a stapler. Attach a red tag to the infected plant with the date of inoculation (Figure 1).

B. Evaluation of cucurbit lines inoculated with *Pseudoperonospora cubensis*

1. Check all inoculated plants first-thing the morning after inoculation. Check for symptoms of downy mildew on the detached leaf, attached and surrounding leaves. Continue to monitor every morning and afternoon.

2. Evaluate plants for 5 days. Results should be recorded as to the percentage of inoculated leaves infected and leaf area infected over all the inoculated plants of each line. (see evaluation sheet).

Orange Tag

Figure 1. Labelling of tags.
Orange tags should be labelled with the line number and the date of inoculation.

Spray inoculation method

A. Inoculation cucurbit lines with *Pseudoperonospora cubensis*

1. First thing in the morning (08:00) on the day before inoculation, check downy mildew infected source material to ensure that material is available for inoculation at 16:00. One well infected leaf is required per 100 ml of inoculum.
2. Determine how many lines need to be inoculated. At least 5 plants from each line need to be inoculated in addition to the positive (???) and negative (Big-C) controls. Only three leaves per plant needs to be inoculated.
3. Prepare sufficient dH_2O (at least 1 litre) and store at 4°C for the following day.
4. At 08:00 of the day of inoculation collect infected leaves. Choose the leaves with large amounts of fungal growth on the underside of the leaves. Maintain them in plastic bags in ice boxes with ice.
5. In the lab, confirm presence of the fungi using the microscope. This is only necessary for one leaf.
6. Place the leaves in a plastic or glass beaker. Pour chilled dH_2O (4°C) onto the leaves. Add approximately 100 ml per leaf.

7. Gently stir the mixture to help release the fungi from the leaf surfaces.
8. Maintain the solution at 4°C.
9. At 15:00, remove the leaf/fungi solution from the refrigerator. Stir for approximately 1 minute. Remove the leaves.
10. Take a 100 μl aliquot and observe under the microscope.
11. Ensure that there are large amount of sporangiophore and sporangia. (NB. We will at some stage compare evaluate which concentration is the best.)
12. Stir the fungal solution and then pour into a screw-top glass or plastic container.
13. Walk to the field or screenhouse.
14. Fill the sprayer ("foggy").
15. Label the leaves of the plants to be inoculated (Figure 1) – 3 leaves per plant. The leaves should be "middle-aged".
16. Spray the leaves.

B. Treatment of inoculation materials

1. At the completion of the inoculation, dispose of the plant material into the pathogen waste container. Material of a quarantine nature, including the plastic bag, should be bagged and autoclaved.

2. The used containers and sprayer should be thoroughly washed and then soaked overnight in 10 ppm chlorine (approximately 0.01 ml of 8.8% Clorox per litre of water).

3. After overnight soaking, the bottles and sprayer should be removed, drained, rinsed with tap water and dried.

C. Evaluation of cucurbit lines inoculated with *Pseudoperonospora cubensis*

1. Check all inoculated plants first-thing the morning after inoculation. Check for symptoms of downy mildew on the sprayed leaves as well as surrounding leaves. Continue to monitor every morning and afternoon.

2. Evaluate plants for 5 days. Results should be recorded as to the percentage of inoculated leaves infected and leaf area infected over all the inoculated plants of each line. (see evaluation sheet).

Preparation of seedlings

Cucurbit seeds should be sown in 10 cm (4 inch) plastic bags containing a 1:1 ratio of potting mix ("Dutch peat") and sand. Seedlings should be approximately 15 days old.

Watering of seedlings

Watering of seedlings should be as required but be consistent – approximately 50 – 100 ml twice per day during the hotter months of March – October and 50 - 100 ml once per day during the months of November – February (this corresponds to the soil on the top of the pot being wet and water running out of the bottom of the pot. Over watering should be avoided.

On the day of inoculation, plantlets should be watered as normal but the final watering MUST BE completed before inoculation.

Materials required: beaker containing bacterial suspension, containers for dipping the seedlings, marker pens, wash bottle containing 70% ethanol for swabbing, paper towels, plastic tags, seedlings (young plants), gloves, large washing tub for washing the used containers overnight with Clorox, fan to keep cool, yellow tags labelled with the plant line, date of inoculation and bacterial isolate and white paper tags labelled with the plant line and bacterial isolate (Figure 3):

A. Positive control - 5 plantlets of the bacterial wilt susceptible line ?
   Negative control – 5 plantlets of the bacterial wilt tolerant line "# ?"
B. Non-inoculated "?" control – 5 plantlets
C. Non-inoculated "# ?" control – 5 plantlets
D. Inoculated sample - 15 plantlets of each line being screened
E. Non-inoculated sample – 5 plantlets of each line being screened

Proceed to the screenhouse.

Transfer plants from the "CLEAN AREA" to the fungi screenhouse.

Check that all pots are correctly labelled with a white tag indicating the line and the date the seed was sown (eg. ?? 22/7/44)

DM Screening Data Recording Sheet

Date seeds sown: **Date of inoculation:** **Date of record:** **Isolate used:**

A. CONTROLS

Line	1	2	3	4	5	AV	Comments

B. TEST

Line	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	AV	Comments