

# Juvenile Hormone treatments on mealybugs

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## Abstract

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## Protocol

### Step 1.

Prepare pyriproxyfen dissolved in methanol.

5mM is used to treat batched of 5 prepupae

20mM is used for single female third-instar nymphs

### Step 2.

Place the individual(s) on a filter paper. For males, brush off the secretions because they will otherwise stick to the cuticle after treatment and lead to higher mortality rates.

### Step 3.

If treating multiple individuals, gather them on a pile using a fine brush.

### Step 4.

Apply pyriproxyfen or methanol on the pile or the tergite of single individuals.

For prepupae, 2-5uL can be applied at once using a micropipette

For females, one single individual can be treated with 0.5 uL applied on the tergite

### Step 5.

The excess solution should be absorbed by the filter paper and evaporate quickly.

After absorption, use a brush to move the individuals away from the treated spot and let them recover (wait for them to start moving again)

## Step 6.

After they start moving, transfer the males in a 1.5 mL Eppendorf tube, after adding a cap filter that was humidified with 10uL of distilled water. Incubate the male in the tube until they reach the desired stage of time for observation, data collecting.

For females, after they start moving again, transfer in a container with a food source (sprouted potato or bean).

## Warnings

Pyriproxyfen dissolved in methanol is volatile and harmful for the environment.

Separate brushes for control and treated samples.

Discard material that touched pyriproxyfen in plastic bags and using the guidelines for cytotoxic waste or similar of your institution.

Wipe surface with 70% ethanol after treatments.