A Detailed Guide for Testing Fruit for the Presence of Spotted Wing Drosophila (SWD) Larvae

Drosophila suzukii

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Growers, backyard gardeners, researchers, fresh market producers, packers, processors, and field scouts need a reliable, quick, inexpensive, and clean method to assess fruit infestation by insects before processing, storing, shipping, or selling fruit.

The following is a 7-step guide for testing fruit for spotted wing drosophila (*Drosophila suzukii*; SWD) larval infestation. This procedure emerged as the most reliable of several methods tested at Oregon State University. The research was conducted in collaboration with the U.S. Department of Agriculture, Agricultural Research Service, Horticulture Crops in Corvallis, Oregon, on blueberries, caneberries, strawberries, cherries, and grapes.

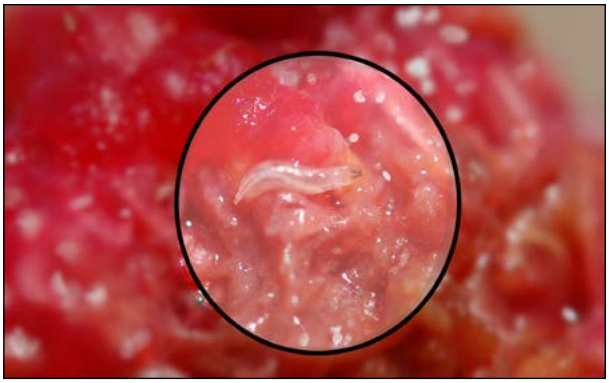

Figure1a. Small white larvae hatch from eggs within a few days and feed inside the fruit, causing it to soften and collapse around the feeding site. The larvae may pupate inside or outside the fruit.

Figure 1b. Spotted wing drosophila (SWD) look like other *Drosophila* in the same family of flies (vinegar or pomace flies, but sometimes called fruit flies). However, SWD prefers laying eggs in healthy fruit as it ripens on the plant versus damaged or decaying fruit.

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Step 1: Gather supplies

Figure 2a. A variety of different-sized trays can be used for examining berries or other fruit for SWD larvae.

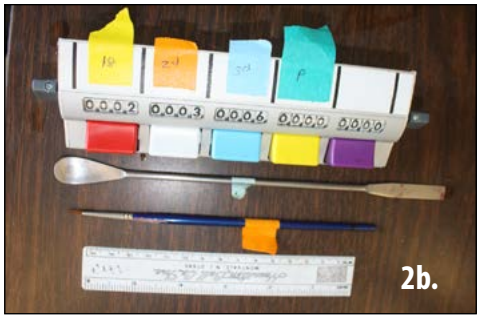

Figure 2b. A counter, stirring stick, tweezers or paintbrush, and ruler will aid the counting process.

Magnification, good lighting are key tools

Collect the following items:

- Plain table salt or light brown sugar
- 1-gallon jug for making solution
- Sealable quart or gallon plastic bags
- White or black shallow tray (e.g. 8" x 10", 9" x 11", or 10" x 13"), with shallow sides (approximately 2.5" or 6 mm high)
- Fine paintbrush
- Forceps or tweezers
- Magnifying headband, such as a 3- to 7-power OptiVisor, or magnifying lens
- Good lighting
- Stirring stick or spoon
- Rolling pin
- Wire 4-mesh screen
- Record book

Step 2: Make solution

Salt or light brown sugar with water makes a good medium

a) Prepare solution: Prepare a salt or brown sugar solution at least the day before usage to ensure that the salt or sugar dissolves completely in 1 gallon of warm water. Add the amount of salt or sugar according to Table 1 and shake well to increase the rate at which the salt or sugar dissolves. A well-dissolved solution increases the chance of larvae floating to the surface of the solution. Shake solution again just prior to use.

Table 1: Amount of salt or light brown sugar to add to 1 gallon of warm water for larvae solution

Plain table salt	Light brown sugar
1 full cup (350 grams)	2.5 cups (650 grams)

b) Differences between light brown sugar and salt solution: Both sugar and salt solutions prompt larvae to exit fruit.

Larvae will float to surface and eventually drown in either solution. However, the sugar solution keeps larvae alive longer, and enables the viewer to see movement on top of the solution for an extended period of time. For those who are just confirming the presence of any type of larvae, the choice between brown sugar and salt is not as critical. If confirmation of species is necessary, larvae can be removed from sugar solution and processed as described under Step 7e.

Brown sugar is a more expensive ingredient than salt, can be sticky and messy, and is an attractant to other insects. The brown sugar solution is darker than a salt solution, making it slightly more difficult to spot larvae through the dark fruit juices. Salt is an irritant to the larvae, causing them to exit fruit quickly, but it kills the larvae more quickly.

Step 3: Collect fruit

Figure 3a. Damaged fruit may have a collapsed side with juice exuding from hole where egg was laid.

Figure 3b. When collecting fruit, it's important to label the sample with the location.

Gather both damaged and healthy-looking fruit

a) Damage symptoms:

Fruit with SWD larvae feeding inside may feel soft, have a dull appearance, or have a collapsed side. There may also be juice exuding from egg-laying holes, a bruised and dull look, and the presence of specks, spots, or pinholes where a female fly laid eggs. The cores of blackberries may look gray and mushy. Some blackberries may appear healthy, especially if SWD is in an early stage of infestation. In raspberries, the receptacle exposed after picking may have red coloration, indicating larval feeding in the berry.

For more details on symptoms of damage, see *Recognize Fruit Damage from Spotted Wing Drosophila* (EM 9021) at extension.oregonstate.edu/catalog/

b) Fruit is more likely to be contaminated with SWD larvae when:

- Fruit is harvested too late in the summer (SWD populations increase as the fruiting season progresses).
- Overripe fruit is left hanging on the plant due to untimely or unfinished harvest or because it is hidden in the canopy and not picked in a timely fashion.

Figure 3c. A fruit grower can open up a piece of suspect fruit and look for larvae. The larvae will move when exposed to sunlight.

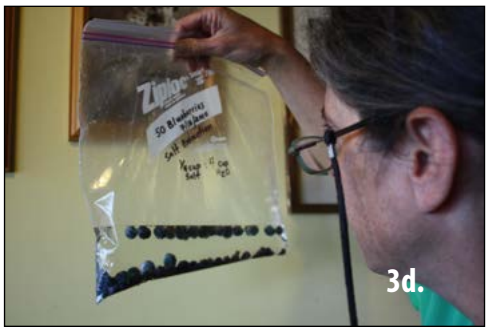

Figure 3d. Another quick method is to crush several fruit and put it into a plastic bag with salt or light brown sugar solution and watch for the larvae to float to the top.

- Fruit is located within protected, shady areas of the plant.
- Farms are diversified with many seasonal fruits.
- Fruit crops are adjacent to diversified landscapes containing non-crop fruit hosts, near places where SWD are protected and can hide and feed, or near a riparian area with high humidity.

c) Sample size guideline:

Field populations of SWD are highly variable, and a small number of samples may miss an infestation. To increase confidence that fruit is reasonably free from SWD infestation, take a larger number of samples (e.g., perhaps 10 to 20 samples) in several "high risk" and other production areas. The more samples the better, but time and cost will dictate the minimum number of samples you need to provide adequate information. The process described here is a guide to testing suspect fruit; it is not a guarantee that you do not have an infestation somewhere in your crop.

Homeowner: A backyard gardener can easily open up a suspect fruit and see 3- to 5-millimeter, whitish larvae with the naked eye. Larvae will move when exposed to light. Alternately, a handful of fruit (e.g. five, 10, or 25 fruits) can be placed in a plastic bag, crushed, mixed with either a salt or sugar solution (see Step 2), and then closely examined for larvae.

Commercial Growers: This group should collect a larger sample (e.g., 100 to 200 fruit) in several areas to help determine if they have an SWD problem. The more fruit they inspect, the more confidence they will have in the quality of the rest of the crop.

Field scouts and researchers: Some have collected 25 to 100 fruits depending on fruit type and fruit size to study levels of damage. No thresholds have been established to correlate adult fly counts found in monitoring traps with larval infestation to help determine the need for treatment.

Processors: Several food-processing industries are testing a minimum of 2-pound samples, representative of each load of fruit.

Step 4: Crush fruit

Figure 4a. You can use your fingers to crush the fruit.

Figure 4b. A rolling pin is another good way to crush fruit.

Crushing fruit will expose more larvae to the solution

Although you can test uncrushed fruit by immersing it in a solution, crushing the fruit helps the salt or sugar solution penetrate the pulp and reach larvae inside. Crushing also increases the number of larvae that float, and forces the larvae out faster than simply placing whole fruit in the solution. Crushing can yield a more accurate assessment than testing whole, uncrushed fruit.

Some cherry industries use a crushing machine. Another method is to use a small rolling pin or just your fingers to mash up fruit stored in a sealable gallon plastic bag. The goal is not to produce a fruit purée, which has pulp strings that make the larvae harder to see, or to use so much force that the larvae are damaged.

Step 5: Add solution

Figure 5a. Add the salt or light brown sugar solution over the crushed fruit.

Figure 5b. Close the bag and swoosh the contents around to circulate the solution.

The liquid should cover the fruit

Pour salt or sugar solution into the bag over crushed fruit. The solution should cover the fruit. Gently shake the closed bag to agitate the fruit and circulate the solution. For a quick look, hold the bag up to the light. If they are present, larvae should be floating on the surface of the liquid along the edges or lining of the

plastic bag. It's also important to stir your solution and crushed fruit to dislodge those larvae that can get caught in and under the fruit skin or pulp, or move to the sides of the tray.

Step 6: Pour into a tray

Figure 6. Pour the contents of the bag into a tray. White trays work well, but some researchers prefer black trays or clear trays that are illuminated from beneath the tray.

Add more solution to the tray if fruit is not completely covered

Empty the berries and the solution into a shallow-sided white tray. A white tray helps reflect light and makes the larvae easier to see in the berry-colored solution. Black trays can provide more contrast, and are preferred by some researchers. Some researchers use clear glass dishes with lighting from below.

Fruit should be covered by ¼ to ½ inch (about 1 centimeter) of solution; add more solution if the fruit is not covered. Fruit should be spread in a single layer with the berries spaced apart for easy viewing and for larvae to exit to the surface. Manually break up any fruit that was not adequately crushed previously.

Sometimes fruit will float, making it difficult to see the larvae. A wire mesh screen can be used to hold down the fruit while giving larvae room to emerge and float to the surface. Submerging fruit will encourage larvae to exit and float, making counts easier and more accurate. A small paintbrush or fine forceps (tweezers) can be used to remove larvae while counting and to get a closer look. Watch for SWD larval movement on the surface of your solution.

Step 7: Examine the solution

Figure 7a. Good light and magnifying lenses help you spot the larvae.

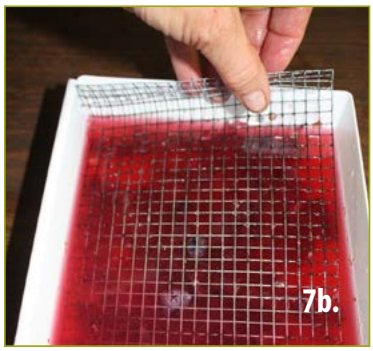

Figure 7b. A wire mesh helps hold down floating fruit pieces.

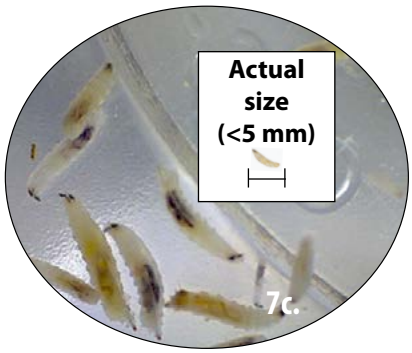

Figure 7c. Magnified images reveal the larvae's pointed black mouthparts, stomach contents, and two tan posterior protrusions.

Most larvae will exit the fruit within 15 minutes

a) A well-lit area is necessary to see SWD larvae and get accurate counts. An overhead light or bright natural lighting will greatly improve viewing.

b) Count larvae immediately—before they die and possibly sink to bottom of tray. Many of the larvae will exit right away; the majority will exit fruit within 1 to 15 minutes. Regularly stir the solution to bring larvae to the surface and to break surface tension around container edges where more larvae may be found. Larvae can be seen with the naked eye, but a magnifying glass or magnifying headset improves your ability to see even the small larvae and their body movements. The more often you sample fruit, and the larger the sample size, the better your chances of discovering the presence of SWD early on.

Larvae are small (typically less than 5 mm, or less than 1/8 inch) and milky white to a glossy, pale-yellow color. The pointed end of a larva (anterior) is darkened, which is the fly's mouthparts with hooks (mandibles). The darkened area within the larva's body is digested berry contents in the stomach. On the posterior end, there are two protrusions on the ventral surface. These protrusions, which are used for moving, vary in size and are not obvious to the casual observer.

Drosophila suzukii have three larval stages, called instars (Figure 8): the first instar, when they are small and young and less than 2 mm long; the second instar, when they are medium-sized from 2 to 3.5 mm long; and the third instar, when they

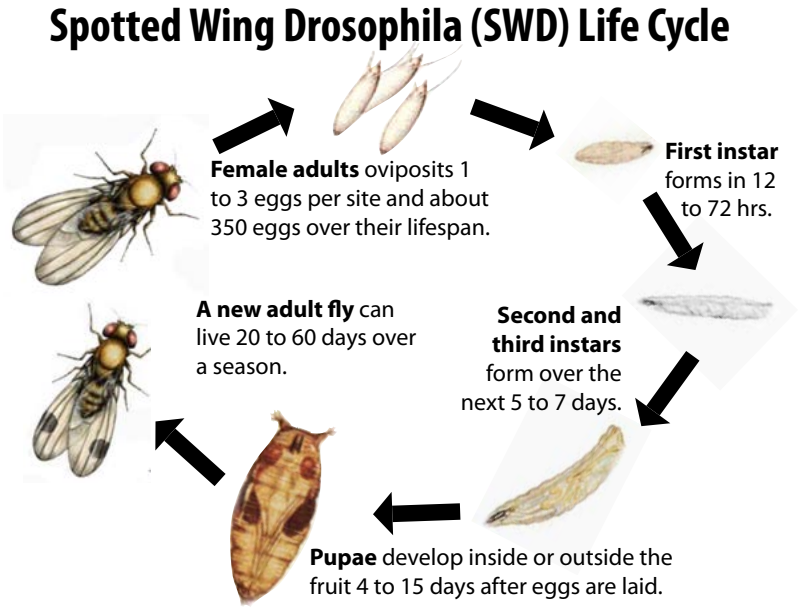

Figure 8. The SWD life cycle, from egg to adult, can happen in 8 to 14 days depending on environmental conditions in the field. Multiple generations are produced each year.

are from 3.5 to approximately 5 mm long. Visible larval size ranges from 0.7 mm to 5.4 mm in length.

c) Record your findings. Begin counting larvae within the first minute of placing solution over crushed fruit. Within fifteen minutes, more than 70 percent of larvae present will have exited. Some larvae can get caught in the berry skin and go unnoticed. It may take longer for small larvae to exit fruit. Closely watch surface of solution to spot the movement of live larvae. While counting, you should:

- **Record numbers of larvae** in a record book. Note the date and location that fruit was picked to help identify high-risk areas for SWD.
- **Look for eggs.** The extraction method will not detect eggs, but in some cases you can see them on the surface of the fruit if you look closely.
- **Stir the pulp.** About 90 percent of larvae should float within sixty minutes of being immersed in the solution, although some will sink or get tangled up in fruit—especially strawberries and blackberries. Stir contents with a stirring rod to help release them. Look closely for floating larvae and ones that may sink or get tangled in fruit.

e) Confirm identification of larvae

For a more thorough examination and to acquire an SWD infestation rate or confirmation of species, the larvae must be reared to adulthood. At this time, there is no other method of determining the species. Here's how to "rear out" the larvae to adulthood:

- Place fruit with larvae in individual plastic cups with ventilated lids, or move larvae with a fine paintbrush to a cup of instant "Drosophila food media" (which can be purchased from online biological supply houses).
- Be aware that mold may develop on fruit, such as strawberries and raspberries, so a screened lid for good air circulation may be necessary, along with a layer of sand in bottom of cup to soak up juice.
- Be sure to secure lid to maintain the moisture for larvae and to prevent escape of the adult fly when it emerges. If humidity is not adequate, SWD development may be affected.
- Store cups at room temperature. Allow up to 14–18 days for larvae to mature to adult flies.
- Some researchers place whole fruit in paper bags at room temperature for approximately three weeks to rear out adult flies and minimize the labor of using developmental cups.
- Others place individual fruit on an elevated screen situated above a layer of sand or a baby diaper placed inside the bottom of a plastic, shoebox-sized container with a ventilated lid. Placing a small section of yellow sticky trap inside of the container will allow you to open the container and examine the trapped flies without the risk of them flying away.

Do not mistake SWD larvae for these contaminants:

- Floating plant parts, such as pistils (stigma and style), achenes (seeds), or corollas
- Thickened pulp pieces/threads, straw, miscellaneous fine debris
- Small narrow thrips
- Fruitworms, sap beetle, or leafroller larvae
- Aphids and aphid skins
- Hover fly (syrphid fly) or lacewing larvae
- Other *Drosophila* larvae

Figure 9. Sap beetle larvae and fruitworms look like SWD larvae.

Get to know the look of *Drosophila* larvae and establish a search image in your mind: (small size, no legs, no head, white to glossy, pale-yellow color with black mouthparts on pointed end; two small knobs for breathing on posterior end; squirming movement) that will help you identify them correctly.

There are other species of *Drosophila* with similar characteristics, but infestations in intact fruit tend to be dominated by SWD. There is no key for distinguishing between different species of *drosophila* larvae. If it is necessary to know the species, raising the larvae to adulthood is the only way to confirm species at this time. For help in adult fly identification, see the Oregon Department of Agriculture SWD key (**oregon.gov/ODA/PLANT_suzukii_id_guide10.pdf**) or watch an Oregon State University identification webinar (**gpdn.org/webinar_2012**).

Figure 10a, 10b. An observer (left) closely examines crushed fruit for larvae. Others (above) hold plastic bags of crushed fruit in solution up to the light to see if larvae are floating on the surface of the liquid.

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