

Protocol for Extracting Rust Mites from Grapevine Tissues

Dr. Patty Skinkis, Viticulture Extension Specialist and Associate Professor, OSU

Dr. Paul Schreiner, Research Plant Physiologist, USDA-ARS

Dr. Amy Dreves, Entomologist, OSU

This user-friendly protocol outlines a method used to remove rust mites, thrips, predatory mites, and other insects from grapevine bud, leaf or stem tissues. Once the sample is collected from the vineyard, this method can be used to wash tissues and count the number of organisms per tissue under a dissecting microscope. You can also view an instructional video online: [http://youtu.be/9Qe4gJ3y -s](http://youtu.be/9Qe4gJ3y-s). See also the supplemental protocol workflow document.

Supplies

- Plastic re-sealable sandwich bags or gallon-size (depending on size of tissue)
- 70% isopropyl alcohol (rubbing alcohol)
- water
- plastic petri dishes (100 mm x 15 mm dish)
- measuring cup or 50 ml graduated cylinder
- tally counter
- scissors
- stereomicroscope/dissecting scope (40X or greater)
- notebook or data collection sheet

Field sampling

What to collect:

- Winter: dormant canes or sloughing bark from the head of the vine trunk or upper trunk
- Spring: emerging buds or whole shoots in spring (best when <6" long)
- Summer: leaves from mid to upper canopy
- Late Summer: leaves from any part of the canopy

How to collect:

- Collect each tissue sample into individual plastic re-sealable bags as outlined in Table 1. Label each bag with date, vineyard block, and company name.
- Do not combine all tissues from your sampling areas into one bag for separation later. Bagging tissues individually allows you to isolate pests per tissue and determining the level of infestation. Also, this bag will be used later for directly washing the sample. Thrips are very mobile unlike rust/bud mites, and bagging the individual leaf or small shoot allows you to capture and count all pests that may be present, not just those that could not escape.
- Store tissues at refrigeration temperature (~40°F) until ready for washing and counting. Mites can remain in refrigeration for several days and up to a week. Longer refrigeration may result in mite death, so make sure to analyze or submit your samples for analysis relatively soon after sampling.

Table 1. Sample collection and handling

Time	What to Sample	Handling
Winter	Dormant canes	Collect dormant canes from individual vines. Place in a plastic bag.
	Bark	Collect sloughing bark from the vine, focusing at the head region. Place in a gallon-size re-sealable bag, keeping vines samples separate.
Spring	Newly emerging buds	Collect newly emerging buds (3-5) per sandwich size re-sealable bag.
	Small shoots (<6")	Whole shoots can be collected in early spring, place 2-3 shoots per sandwich size re-sealable bag if they are ≤ 3 " in length. If the shoots are 4-6" in length, bag them separately.
	Leaves	If shoots are >6" in length, collect leaves and place several leaves (2-3) per individual plastic bag. Focus attention on lower leaves on the shoot.
Summer	Leaves	Collect leaves and place each leaf in its own plastic bag. Do this immediately upon collecting the leaf so as to capture more mobile pests.

Extraction and Counting Procedure

1. Dilute the 70% isopropyl alcohol to 35% by combining one part alcohol and one part water. Store in a glass or plastic bottle until ready to use.
2. Prepare the tissue for washing according to the type of tissue (Table 2).

Table 2. Sample preparation

Tissue	Preparation technique
Leaves	Cut the leaf into small pieces of ~ 1" squares and place back into the sandwich bag used to collect the sample.
Shoots (<6" in length)	Remove the leaves from the shoot and cut up the leaf and shoot tissue into ~1" pieces and place back into the sample bag.
Newly emerging buds	These very small (shoots <1") should be cut up in small pieces (2-3 cuts per shoot) and placed back into the sample bag.
Bark	Cut the bark pieces into ~1" pieces using a pruning shears. Place 5-6 pieces into a sandwich size re-sealable bag for the washing step 3.
Dormant canes/buds	Cut buds off the cane using a pruning shears. Slice the bud into pieces using a razor blade, and place all buds from your cane sample into a sandwich size plastic bag or blender for the alcohol extraction step 3.

3. Add ~45-50 ml* of 35% isopropyl alcohol into the bag with the leaf pieces and seal closed.

**You may need to adjust the amount of alcohol based on the size of the petri dishes used. Approximately 45-50 ml of solution will fit into the bottom dish of a 100 mm (diameter) x 15 mm (height) petri dish. Also, 45-50 ml is adequate to provide enough coverage while washing an average fully-expanded leaf during summer.*

4. Shake the bag vigorously for 30 seconds or more, cut the corner with a scissors and allow the alcohol to flow into a petri dish, leaving behind the pieces of tissue. The sample is now ready to view under a dissecting microscope for counting. If you are working with dormant bud tissues, place the tissues into a blender with the alcohol and blend on low speed for 1 minute. Pour off the alcohol into a petri dish, leaving behind all plant tissues.

Observing under the microscope

- Most insects will sink to the bottom of the petri dish and be relatively easy to view. A few of them may remain floating in an aggregate at the top of the sample. Make sure to alter the view to count both those that are sunken and those that are floating. Magnification of greater than 45X is needed to see the mites in the sample.
- It is best to use both over- and under-lighting on the dissecting microscope if it is equipped with both lights.
- If you want exact numbers of mites present, use a petri dish with etched lines on the bottom or simply draw lines on the bottom at a width of 5 mm (1/4 inch) between lines using a thin permanent marker (Figure 1). Be sure not to move the petri dish too quickly so as to lose count or redistribute mites. Use a counter to tally the number of mites you observe.
- If you do not need exact numbers but want to know a general range of mites present, take the time to train yourself by counting a few samples to gauge what hundreds or thousands of mites in a sample look like. After a while, you will easily be able to determine the generalized quantity of mites in a sample.

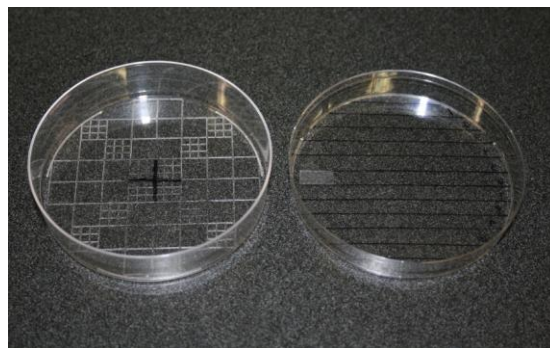


Figure 1. Petri dishes with etched grid marks (left) or grid lines drawn with permanent marker on the exterior (right) will make counting mites much easier. *Photo by Patty Skinkis, OSU*



Figure 2. Rust mites may appear clear, tan, amber or milky white, depending on stereoscope lighting and the life stage of the mite. Both images shown were photographed from a compound microscope but can be useful in identifying the shape of the mites. *Photo by Paul Schreiner, USDA-ARS*



Figure 3. This is an image of a predatory mite. They will look more translucent in the wash and will be visible at a much lower magnification than the rust/bud mites. You can count these and thrips at a lower magnification first then progress to a higher magnification for rust/bud mite counts.



Figure 4. Rust mites are much more difficult to find on tissues, particularly in spring. Mites have an elongated shape similar to a rice grain and may be tan, off white, milky white, translucent or darker brown, depending on life stage when observed under a stereoscope. This image shows rust mites on leaf tissues collected in spring. *Photo by Paul Schreiner, USDA-ARS*



Figure 5. Thrips are much larger than rust mites and can be found moving quickly on leaf surfaces. Using the bagging method helps get a better estimate on their presence because it captures them before they escape the leaf/other tissue. Thrips are much larger than rust mites and can be seen at a lower magnification. Therefore, it is best to count them and predatory mites before advancing to higher magnification to count rust/bud mites. *Photo by Paul Schreiner, USDA-ARS*

More information

For more information about this method and grape rust mites, please see the publications and resources below:

Schreiner, R.P., P.A. Skinkis, and A.J. Dreves. 2014. A rapid method to assess grape rust mites on leaves and observations from case studies in Western Oregon vineyards. *HortTechnology*. 24: 38-47.

Skinkis, P.A., J.W. Pscheidt, E. Peachey, A. Dreves, V.M. Walton, D. Sanches, I. Zasada, and B. Martin. 2014. 2014 Pest Management Guide for Wine Grapes in Oregon. OSU Extension Publishing. <http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/45975/em8413.pdf>

Skinkis, P. 2014. Grape Rust Mites, eXtension/eViticulture.org. http://www.extension.org/pages/33107/grape-rust-mite#.U_yZChcXOV0

Skinkis, P., J. DeFrancesco, and V. Walton. 2014. Grape Rust Mite, PNW Insect Management Handbook. <http://insect.pnwhandbooks.org/small-fruit/grape/grape-grape-rust-mite>