

# FACT SHEET

National Clean Plant Network



Start clean, stay clean.



## VIRUS ELIMINATION using MICROSHOOT TIP THERAPY

Virus, viroid and virus-like diseases are some of the most important production constraints in vegetatively propagated crops such as berries, citrus, fruit trees, grapes, hops, roses and sweetpotatoes. Plants may become infected with these pathogens by poor propagation practices or in the field. Once a plant is infected with viruses, viroids or virus-like diseases, most cuttings taken from the plant carry them as well.

Several types of therapies are used to eliminate viruses and viroids from a plant. They include: microshoot tip culture, meristem culture, embryogenic culture and micrografting. These therapies may also be combined with heat, cryogenic or chemotherapy. Microshoot tip therapy is one of the most reliable methods and has been used for decades on a wide range of ornamental and crop plants.

### What is a microshoot tip?

A microshoot tip consists of the apical meristem, a dome shaped area at the growing tip of a shoot that contains a few hundred undifferentiated cells, and 2 to 3 pairs of leaf primordia. A microshoot tip is 0.2 to 0.5 mm in size.



*A microshoot tip of a grape showing the dome shaped apical meristem and several pointed leaf primordia.*

### What is microshoot tip therapy?

Microshoot tip therapy is the process of culturing microshoot tips from an infected plant to generate a population

of new plants using tissue culture techniques. The new plants are established in a greenhouse and extensively tested for viruses/viroids. If no pathogens are detected in a plant, it is used as a source of clean propagation material.

### How is microshoot tip therapy done?

Shoot tips that are about 2 to 3 cm long from a vigorously growing plant are harvested into a humid box and taken into the lab. The microshoot tip is excised in sterile



conditions using a microscope in a laminar airflow hood. To accomplish this, outer leaves are carefully removed until the meristem dome is exposed. The dome and a few leaf primordia are excised and placed on growth media, or, in the case of citrus, the stem of a nurse plant already growing in a tube.

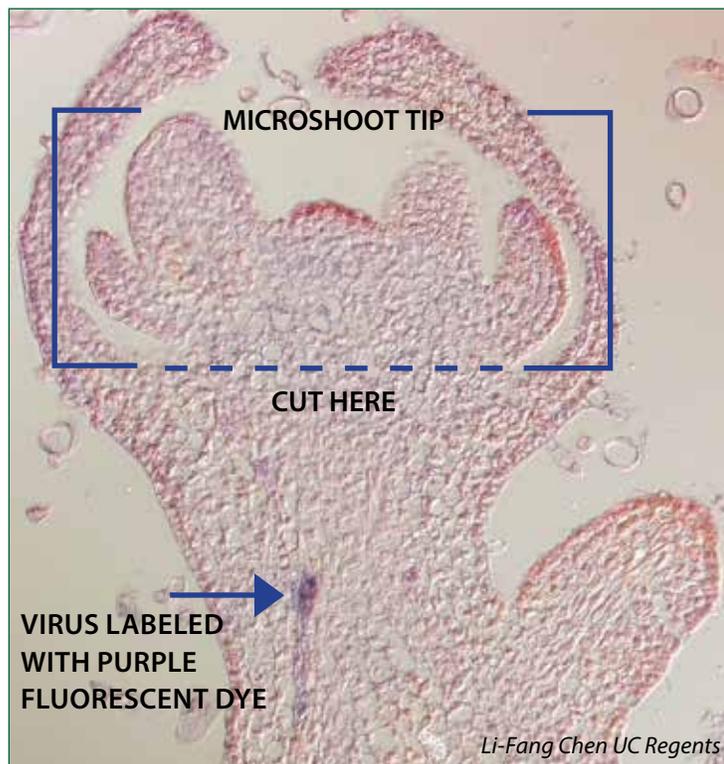
### How does microshoot tip therapy work?

It is not known exactly how microshoot tip therapy works but there are several plausible theories. One theory is that the virus has not yet infected the cells in the growing tip because the plant cells are dividing faster than the virus can replicate and infect. In other words, the plant is growing faster than the virus.

Another theory is that, by chance, the virus has not infected the microshoot tip. Often viruses are unevenly distributed in a plant and some buds are uninfected. If an uninfected bud is selected, the resulting plant will be clean.

Perhaps it works because there is no vascular connection between the youngest part of the tip and the rest of the plant, so the virus is unable to reach the cells at the tip. This is likely the case for phloem-limited viruses (see figure below).

There is also evidence that gene silencing mechanisms may be involved in excluding viruses from the meristematic area.



*In this cross section of a virus-infected growing plant shoot tip, the virus is located in the protophloem cells of the vascular system. Since there is no vascular connection to the microshoot tip, the virus has not infected the cells near the tip. Many important viruses are phloem-limited, and treatment is often more successful with these viruses*

## How successful is microshoot tip therapy?

Overall microshoot tip therapy treatment success is often over 85%, but it varies with crop species, cultivar and virus.

Some crop species and cultivars are recalcitrant to tissue culture; and once in tissue culture, some viruses are difficult to eliminate. Viruses that move readily from cell-to-cell can be very challenging to eliminate. For example, to eliminate raspberry bushy dwarf virus in *Rubus* spp. a combination of therapies and/or many repetitions are needed.



*Grapes take an average of 7 months to grow from a less than 0.5 mm microshoot tip to a 6.0 cm plantlet. If the resulting plant is infected, the process is repeated. In berries, each cycle can take 1 to 3 years.*

Repeated testing after treatment is always necessary to ascertain whether the treatment successfully eliminated the virus.

## Does microshoot tip therapy lead to unintended effects?

Microshoot tip therapy for virus elimination has been used successfully since the 1960s in many crops with no off-type development or other unintended effects. This is because conditions that may lead to negative effects, such as hormones in growth media and time in culture, are minimized. In addition, since the apical bud is excised along with primordial leaf tissue, cell differentiation has already begun and callus cell development does not occur.

Microshoot tip therapy is often confused with tissue culture propagation. Tissue culture propagation involves using larger pieces of tissue, mass production, many subcultures and often many years in culture, which can lead to undesirable traits in plant progeny. Finally, tissue culture propagation does not eliminate viruses.

Short videos showing the excision process under the scope for several crops are available at <http://fps.ucdavis.edu/fgrintroservices.cfm>.

## ABOUT NPCN

The NPCN is an association of clean plant centers, scientists, regulators and growers concerned with the health of specialty crop planting stock.



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[nationalcleanplantnetwork.org](http://nationalcleanplantnetwork.org)

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