

## PRODUCTION OF TREES MODIFIED BY Tsn-RNA's

The following is the step by step procedure for the handling and production of trees modified by the Tsn-RNA: (Please see **Caution** below)

1. Propagate a tree on any rootstock. The scion variety of this tree must be the variety that you will want to use to make modified field trees. This tree can be on any rootstock including but not limited to citrange, trifoliolate orange, volkameriana, rough lemon etc. **This tree will become your mother tree for buds containing the modifying Tsn-RNA that you will use to make your future modified field trees.** If you are going to want to produce more than the one particular variety of modified field trees, then **you must make a mother tree of each variety** that you are going to want to propagate for the field.
2. When the mother tree(s) propagated in step 1 is/are growing well approximately 1-1½ years, order the Tsn-RNA inoculum of your choice from the CCPP. Inoculate the small mother tree with the Tsn-RNA inoculum by placing two separate pieces of the green bark from the inoculum stick under the bark of the scion on the small mother tree. This inoculation procedure is the same as if you were budding a tree except you will not be using a bud but a piece of bark. Make a "T" cut and slip the bark tissue between the bark and the wood, and then wrapping with budding tape.
3. Between 6 and 12 months after you have inoculated your mother tree contact the CCPP so that your tree can be checked for the presence of the proper Tsn-RNA. At the time you contact the CCPP you will be given instructions as to what type of material to send for the test. At this time also you will be assessed the \$250 charge for the testing procedure for each mother tree.
4. You will be informed of the test results as soon as they are completed. Your young mother tree can then be used as a source of budwood for the production of field trees of the same variety as this mother tree. Since the tree will still be very small you will want to allow the tree to grow for at least an addition 2-4 years before using it as a source of budwood. Remember, before this tree may be used as a scion source tree **it must be entered in the CDFA tree registration program** and tested for CTV and any other disease testing that may be required.
5. Once you are ready to propagate field trees containing the growth modifying Tsn-RNA, remember that you will be using the **buds from the registered Tsn-RNA containing mother tree.** In order for the growth modification to occur, **you must propagate the field trees on trifoliolate rootstock only.** Trifoliolate hybrids like citrange will not respond to the Tsn-RNA only Trifoliolate orange such as Rubidoux trifoliolate, Pomeroy trifoliolate, Rich 16-6 etc.
6. Buds from the Tsn-RNA mother tree can only be used to produce field trees on trifoliolate orange and these buds may not be increased by using them in an increase block.

**Caution:** Please remember that these Tsn-RNA's can be spread on budding and pruning tools and equipment. Therefore proper sanitation and handling should be practiced. Budding knives and pruning shears should be disinfected before and after use with your Tsn-RNA trees in a solution of approximately 2% hyperchlorite (normal household bleach at a 1:1 dilution with water).

# Tsn-RNA Use Flow Chart

