NAPPO
North American Plant Protection Organization
Organización Norteamericana de Protección a las Plantas
MEXICO - USA - CANADA

# NAPPO TREATMENT PROTOCOLS

## TP 01
## THERMOTHERAPY OR THERMALTHERAPY

The Secretariat of the North American Plant Protection Organization
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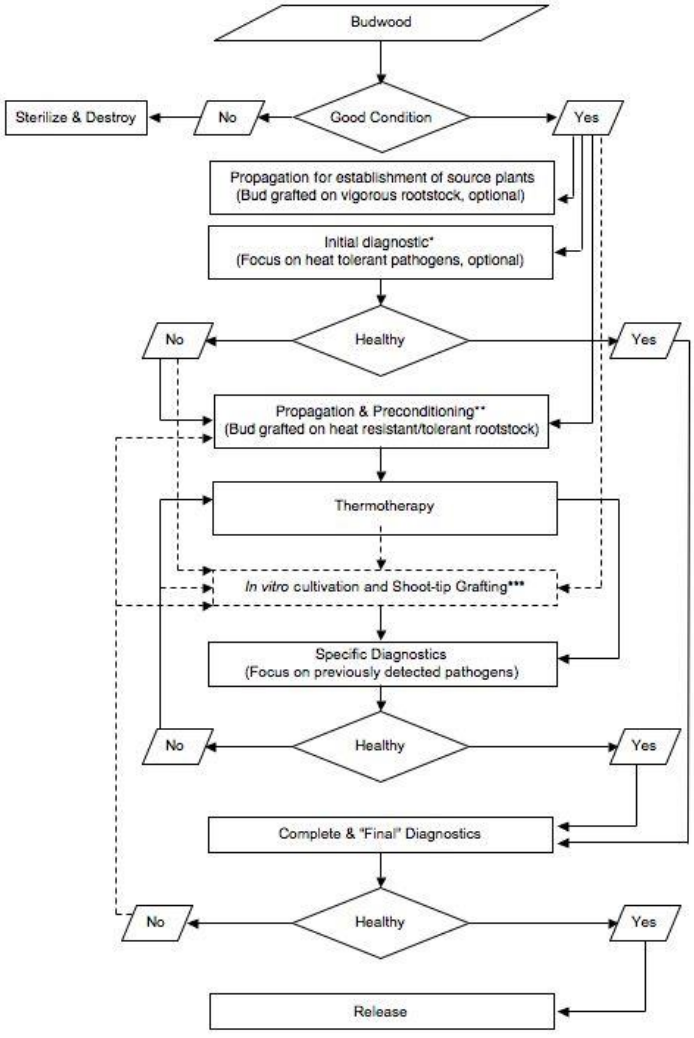
| Active ingredient | NA |
| Treatment type | Thermal: high temperatures |
| Target pest | Virus: *Citrus tristeza closterovirus*, *Citrus psorosis virus*, *Citrus variegation ilarvirus*, citrus vein enation graft-transmissible agent, citrus tatter leaf virus/citrange stunt virus (= *Apple stem groove virus*), other citrus viruses, virus-like, and graft transmissible agents. |
| Target regulated articles | *Citrus* spp. and hybrids |
| Treatment schedule | Thermotherapy is carried out as indicated by pathogen testing results. The procedure requires 2 – 4 weeks of pre-treatment and 12 – 16 weeks of the actual thermotherapy (see protocol). |
| Other relevant information | Thermotherapy treatment should be used as part of the measures applied in a certification program for propagative material free of pathogens. The following chart represents the normal flow of the procedures used for thermotherapy and shoot-tip micrografting treatments:<br>  <br><br>*An initial diagnosis is recommended. This will help to compare the phytosanitary condition of the material before and after the treatment for those pathogens that were detected (not all pathogens are detected with the initial diagnosis). Nevertheless, the propagated material** can be directly subjected to thermotherapy and/or shoot-tip micrografting. |

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| | ***Thermotherapy and shoot-tip micrografting can be complementary treatments. The plant material can be either subjected to both treatments or to one or the other. This protocol refers specifically to thermotherapy. |
| **References** | Brown L. G., L. L. Breman. 1996. Introduction of Citrus Germplasm into Florida. Plant Pathology Circular No. 379. Fla. Dept. Agric. & Consumer Services.<br><br>Calavan E.C., C.N. Roistacher, E.M. Nauer. 1972. Thermotherapy of citrus for inactivation of certain viruses. Plant Disease Reporter 56:976-980.<br><br>Fifaei R., B. Golein., H. Taheri, Y. Tadjvar. 2007. Elimination of Citrus Tristeza Virus of Washington Navel Orange (*Citrus sinensis* (L.) Obeck) Through Shoot – Tip Grafting. Citrus Research Institute, Ramsar – Iran.<br><br>Frison E., M. Taher. 1991. Technical Guidelines for the Safe Movement of Citrus Germplasm. FAO/IBPGR.<br><br>Hiroyuki Ieki, Shun – ichi Yamada. 1980. Inactivation of Citrus Tristeza Virus (CTV) with Heat Treatment: Heat Tolerance and Inactivation of CTV on Rootstock – Scion Combinations. p. 20 – 24. In: Proc. 8th Conf. IOCV., IOCV, Riverside.<br><br>Koizumi M. 1984. Elimitation of Tatter Leaf – Citrange Stunt Virus from Satsuma Mandarin by Shoot – Tip Grafting following Pre – Heat – Treatment. p. 229 – 233. In: Proc. 9th Conf. IOCV., IOCV, Riverside.<br><br>Roistacher C. N. 1993. Psorosis a – Review. p.139 – 162. In: Proc. 12th Conf. IOCV., IOCV, Riverside.<br><br>Roistacher, C.N., and E.C. Calavan. 1974. Inactivation of five citrus viruses in plants held at warm glasshouse temperatures. Plant Disease Report 58:850-853.<br><br>Roistacher C.N. 1977. Elimination of citrus pathogens in propagative budwood. I. Budwood selection, indexing and thermotherapy. Proc. Intl. Soc. Citriculture. 3:965-972.<br><br>RSPM 16. 2013. *Integrated Measures for the Movement of Citrus Propagative Material*. Ottawa, NAPPO.<br><br>Wisler G. C., Brown L. G., Schoulties C. L. 1996. A Manual for Introduction of Citrus Germplasm into Florida. Fla. Dept. Agric. & Consumer Services.<br><br>Zhang T. 1996. Effective Methods for the Elimination of Citrus Tatter Leaf Virus by Thermotherapy and Shoot – Tip Grafting. p. 310 – 312. In: Proc. 13th Conf. IOCV., IOCV, Riverside. |

| **Feasibility and applicability** |
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| **Procedure for carrying out the phytosanitary treatment** |
| **Budwood:** First, the budwood is inspected to make sure that it is suitable for thermotherapy treatment. If it shows signs of tissue oxidation, decomposition, or infestation by fungal plant pathogens, it should be sterilized (e.g. using an autoclave) and then destroyed. |

Budwood must be disinfected by immersion for 10-15 minutes in a 0.5% sodium hypochlorite solution, with moisturizing agent at 0.1% (Tween 20). Rinse three times with sterile distilled water and air dry thoroughly.

From each budstick one or two buds are grafted on a rootstock tolerant to heat, such as 'Carrizo' or 'Troyer' citrange, approximately 4-9 months old or 1 cm in diameter, by making a T cut in the stem of the plant at 10-20 cm from the soil surface. The bud should be removed from the budstick using a diagonal cut, then grafted into the T cut. The graft is tightly wrapped with plastic grafting tape, making a humid chamber to avoid dehydration and flushing. Disinfect the grafting knife between cuts with 2% sodium hypochlorite.

**Treatment:** Budded plants are first preconditioned either in a warm greenhouse room at 32-40 ºC (day) and 26-30 ºC (night) for 2-4 weeks prior to placing them in a growth chamber, or they may be preconditioned in the growth chamber itself with gradual increase of the temperature (starting at 30-32 ºC and increase 1 degree per week) for 4-6 weeks. The grafted plants are then placed in growth chambers with fluorescent and incandescent lights (intensity of approximately 2000 lux 1 m from the lamps) and a relative humidity of 50-60 % (if the relative humidity is regulatable). Most citrus viruses are successfully eliminated when the grafted plants are maintained at 40 ºC/30 ºC (16 hr/8 hr day/night) for a period of 8-12 weeks. However, some difficult to eliminate viruses, such as Dweet mottle graft-transmissible agent and citrus tatter leaf virus, may require additional treatment at higher temperatures or the use of alternative therapy such as shoot tip micrografting. The appropriate regime in these cases is generally 40 ºC/30 ºC for 8 weeks, followed by two additional weeks at 42 ºC/30 ºC, and a final two weeks at 44 ºC/30 ºC (all 16 hr/8 hr day/night).

**Post-treatment:** After thermotherapy, the buds are forced by bending the rootstocks over. In some cases, the buds may be forced while the plants are still in the growth chamber (4 -5 weeks). The forced buds are initially trained to a single leader. Growth from the forced buds provides tissue for diagnostics.

**Diagnostics:** Diagnostic tests should be performed on the material that has been subjected to the thermal treatment in order to corroborate its phytosanitary condition. A number of methodologies can be used for this aim. In a plant certification program biological diagnosis are performed through indexing (i.e. grafting of the treated material in different plants used as indicators of disease). Other methodologies include serological and molecular techniques. RSPM 16: 2013 presents in Annex 1 and 2 the accepted diagnostics tests for the different pathogens that infest citrus.

Plants that have been subjected to thermotherapy are generally indexed or tested for the specific pathogens of interest after thermotherapy. Plants that test free of these specific pathogens are candidates for a full index/testing. If plants test free of one specific pathogen of interest but not of others, additional thermotherapy or shoot-tip micrografting is necessary.

(see flowchart)

| **Feasibility and applicability** (Information should be provided where appropriate on the following items) |
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| **Cost of typical treatment facility and operational running costs if appropriate** |
| To apply the thermotherapy treatment, greenhouses with controlled temperature and growth chambers that reach 45º C temperatures are needed. |
| **Commercial relevance, including affordability** |

Diseases caused by virus, viroids and other graft transmissible organisms cause significant economic losses in citrus production worldwide. Some diseases kill the plant and others decrease production and fruit quality, causing loss of vigor and decreased plant longevity. Viruses and other causal agents of diseases may be transmitted through bud grafting and remain latent (no symptom or disease expression) on the plant for many years therefore, vector and/or additional graft transmission may occur since buds obtained from a plant with latent disease will produce diseased plants.

Countries with modern citrus production have been successful applying certification programs that use plants certified free of diseases, mainly those caused by viruses or similar organisms. These disease free plants are obtained from citrus varieties that have desirable agronomic features but in many cases have been affected by one or more pathogens. For this, techniques that allow obtaining virus and virus like free plants from infected plants are used. Thermotherapy is such a technique used to achieve this objective.

Thermotherapy and shoot-tip micrografting are generally carried out by specific entities charged with providing clean-stock material, such as certification programs. Thus, affordability of the process is spread over the entire group of producers that use materials provided by the program (assuming that it is supported by the producers).

**Extent to which other NPPOs have approved the treatment as a phytosanitary measure**

Countries with a certification program for propagative material such as Brazil, Spain, USA, etc., use thermotherapy in their clean plant programs.

**Availability of expertise needed to apply the phytosanitary treatment**

- Knowledge of heat resistant varieties suitable as rootstock
- Physiology of the plant to establish appropriate pre-conditioning periods
- Knowledge or expertise on diagnostic tests such as indexing, serology, and molecular techniques.

**Versatility of the phytosanitary treatment**

The treatment schedule must be strictly applied.

| **The degree to which the phytosanitary treatment complements other phytosanitary measures** |
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| Thermotherapy is a complementary technique to shoot-tip micrografting. Both are used in a clean plant programs for commercially important plants. By combining thermotherapy and shoot-tip micrografting of apical meristems *in vitro*, it is possible to obtain virus and other pathogen free citrus plants. |
| **Summary of available information of weaknesses of the treatment or potential undesirable side-effects** |
| Thermotherapy *per se* cannot eliminate 100% of the virus that may be on a plant and is not recommended to eliminate viroids or spiroplasmas. |
| **Applicability of treatment with respect to specific regulated article/pest combinations** |
| Thermotherapy is known to be effective against several viruses such as: *Citrus tristeza closterovirus*, *Citrus psorosis virus*, *Citrus variegation ilarvirus*, citrus vein enation graft-transmissible agent, citrus tatter leaf virus/citrange stunt virus (= *Apple stem groove virus*), other citrus viruses, virus-like, and graft transmissible agents. |
| **Technical viability** |
| This treatment is accepted and is applied extensively at the level of introduction and sanitation. |
| **Phytotoxicity and other effects on the quality of regulated articles, when appropriate** |
| There are no phytotoxic damages using the treatment schedule established. |
| **Consideration of the risk of the target organism having or developing resistance to the treatment** |
| Low risk of resistance development to thermal treatment. |

## Review

NAPPO Treatment and Diagnostic Protocols are subject to periodic review and amendment. The next review date for this NAPPO protocol is 2020. A review of any NAPPO protocol may be initiated at any time upon the request of a NAPPO member country.

## Approval

This protocol was approved by the North American Plant Protection Organization Executive Committee on October 19, 2009, and revised on August 3, 2015; it is effective from this date.

Approved by:

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