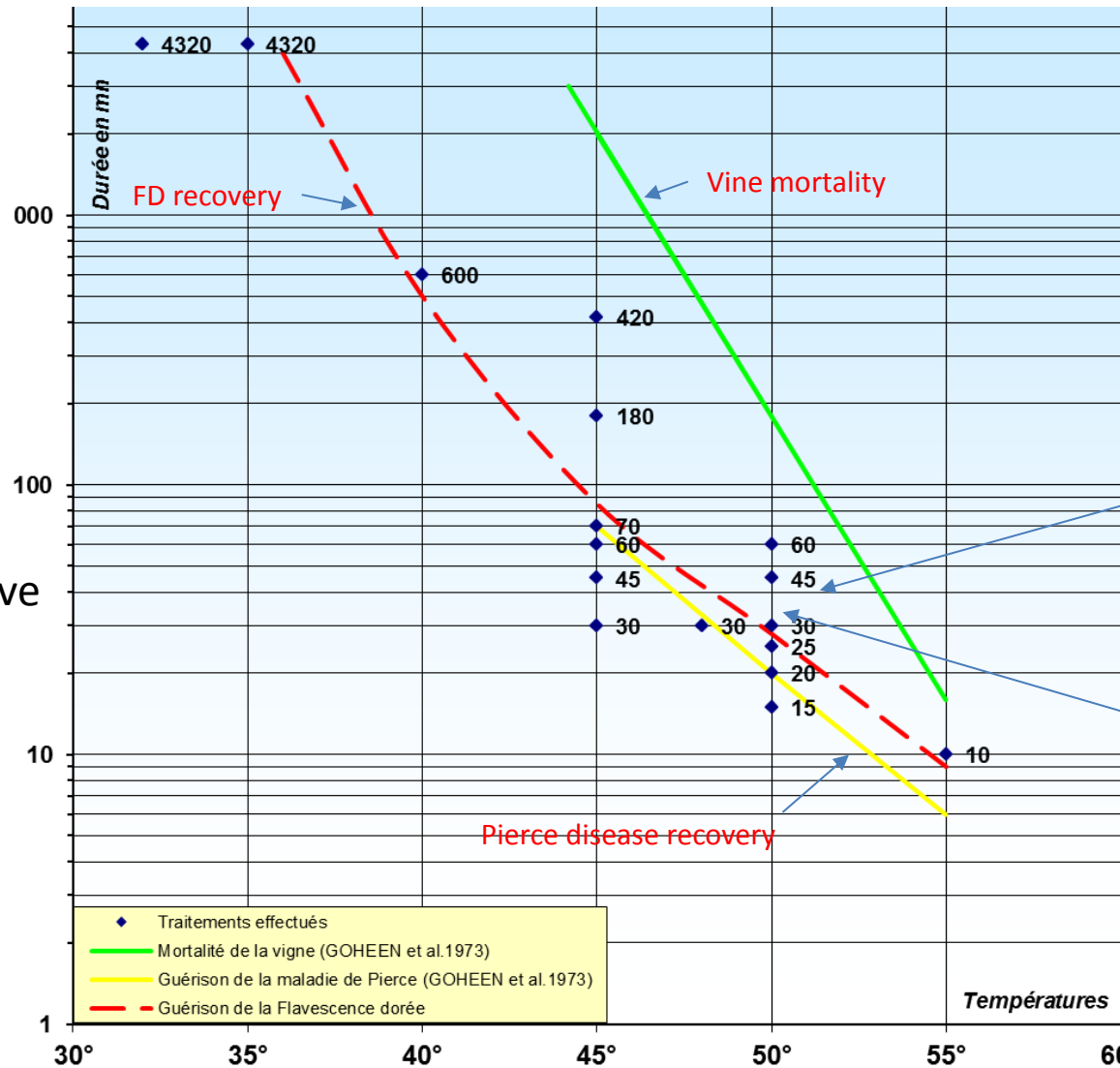


# Hot Water Treatment trials Cuttings and plants

*Modification of the treatment protocol  
duration-temperature*

# Hot Water Treatment efficiency curve

Goheen's curve



Official protocol

Possible margin

# HOT WATER TREATMENT

## Purpose:

Identify an other protocol temperature-duration with the same efficacy against phytoplasma but at a lower intensity than the official protocol (50°C-45')

## Previous trials results:

Effective protocols

Untreated control

Plants GS		tnt	45°-60'	48°-30'	50°-15'	50°-20'	50°-25'	50°-30'	50°-35'	50°-45'
2000: (50/lot) <i>Entav-Inra</i>	FD an1	12%	6%	6%		0%				0%
	FD an 2	4%	0%	3%		3%				0%
2001: (60/lot) <i>Entav-Inra</i>	FD an1	24%			4%		2%	0%	0%	0%
2002: (250/lot) <i>Entav-Inra</i>	FD an1	5%						0%	0%	0%
2002: (300/lot) <i>CA11</i>	FD an1	1%						1%		0%

# HOT WATER TREATMENT

## New Trials in 2011

Untreated control

Résultats PCR1	BOUTURES POSITIVES (Lot 1)						BOUTURES NEGATIVES (Lot 2)					
	213 soit 38%						343 soit 62%					
Modalités de TEC	TNT	50°- 30'	50°- 35'	50°- 45'	48°- 35'	48°- 45'	TNT	50°- 30'	50°- 35'	50°- 45'	48°- 35'	48°- 45'
Boutures traitées	34	34	34	43	34	34	56	58	58	57	57	57
Résultats PCR2 bt positives	20	18	14	20	16	22	-	-	-	-	-	-
soit en %	59%	53%	41%	46%	46%	65%	-	-	-	-	-	-

- 556 cuttings taken on vines with symptoms
- 4 varieties concerned: Alicante HB, Cabernet-Sauvignon, Carignan, Sauvignon
- Individual PCR tests on the 556 cuttings. Only 38% positive → uncertain distribution of phytoplasma and/or lower concentration in the cuttings. Tests more efficient on leaves.
- Tests PCR done after TEC : not conclusive → phytoplasma detection possible even if killed by HWT
- Necessity to visually check symptoms after propagation under insect-proof greenhouse.
- Tests PCR on leaves for all the cuttings

## TRIALS EFFICACY 2011



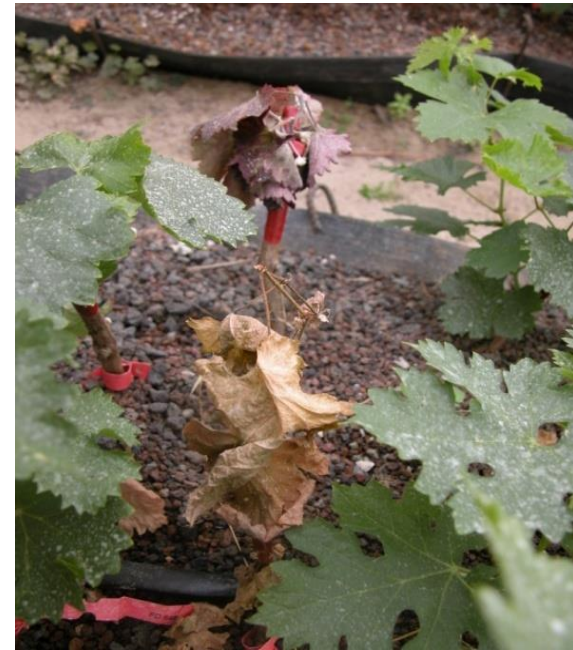
**Trials under insect-proof green house**



# TRIALS EFFICACY 2011



Control plants



## Results: *(batches of 90 cuttings)*

	Controls	48°-35'	48°-45'	50°-30'	50°-35'	50°-45'
% live cuttings	43	76	75	80	74	80
% cuttings FD+ (on 90 cuttings)	<b>17</b>	0	0	0	0	0

Number of live cuttings significantly higher on treated cuttings (HWT curative effect?)

No difference between treated batches

No symptom on treated batches

Results confirmed by PCR tests on leaves

Many symptoms on untreated batches

Presence of phytoplasma confirmed by PCR tests

But results to be verified the following growing season (incubation period?)

- Observations during the second year (cuttings treated in 2011:

Year	Untreated control (cuttings numbers)		48°-35'	48°-45'	50°-30'	50°-35'	50°-45'
2011	<b>15 FD</b>	39 « healthy »	/	/	/	/	/
2012	14: dead <b>1 : FD</b>	<b>5 FD</b>	/	/	/	/	/

Still no cuttings with symptoms on treated batches  
Incubation confirmed on control cuttings  
But no new cuttings with symptoms in 2013





➤ Additional modalities tested in 2012:

Phytoplasma	Control	48°-30'	48°-35'	48°-45'	49°-30'	49°-35'	50°-30'	50°-35'	50°-45'
FD (160 ct/batch) 5 varieties	2%	0%	0%	0%	0%	0%	0%	0%	0%
BN (55ct/batch) Chardonnay	7%			4%			2%		0%

**Bois noir**

- High mortality for all the cuttings (>50%)
- FD: no symptom on treated batches
- FD: 2% of cuttings with symptoms on control plants
- FD: cuttings observation during 2 years after treatment: no new symptoms
- 50°-30' ineffective for Bois Noir
- Might be useful to test 50°-35' for Bois Noir detection



## CONCLUSIONS TRIALS EFFICACY 2011 - 2012

- Confirmation that HWT official protocol 50° - 45' : effective to kill FD and Bois Noir phytoplasma
- Other protocols temperature – duration : effective against FD phytoplasma but lower margin. Well-adjusted machine essential
- Efficacy of Protocol 50°C-35' to be tested for BN

**But before moving forward, what operating procedures in France and in other countries?**

**HWT protocol :** 50°C-45'

**HWT machine identification:**

- Commissioning date
- Maximum capacity of treatment
- Identification number

**HWT machine description:**

- Thermal insulation
- Heating appliance : homogeneous and stable temperature (variations  $< \pm 0,5^{\circ}\text{C}$  during the treatment). Regular sensors verification and calibration
- Automatic recording system
- Time stamp
- Easiness to record movements (dipping and removal)



Technically very difficult to guarantee

## Data recording:

- Temperature and duration
- Machine connected to a secured edition device

## Vegetal material traceability:

- Vegetal material provider
- Vegetal material characteristics

## HWT certificate:

- Baths identification by code number (date, order n°)
- Certificate model

## Data archiving:

- Maintenance of records per campaign
- Data archiving during 5 years

## Technical recommendations:

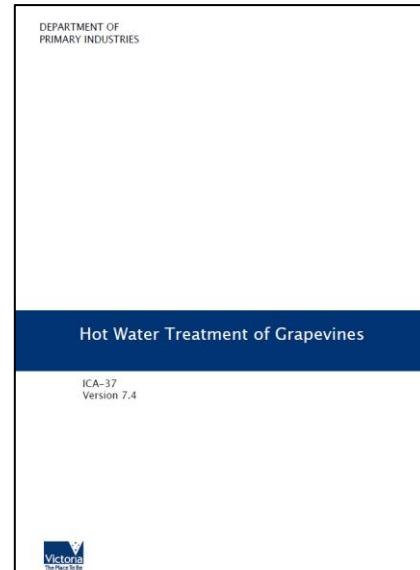
- Storage before and after treatment
- Acclimatization
- Customer information



DEPARTMENT OF PRIMARY INDUSTRIES  
ICA-37 Version 7.4 HOT WATER TREATMENT OF GRAPEVINES

## Highly coordinated procedure in terms of regulatory measures:

- Recommended device, pump, sensors, data recorder, calibration,...
- Sensor's location (3 sensors minimum), operational tests
- Treatments recordings
- Material vegetal traceability before and after treatment
- Treatment station approval process



Probably the most complete

## Treatment protocol:

- $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$  during 30 minutes (option 1) or  $54^{\circ}\text{C} \pm 1^{\circ}\text{C}$  during 5 minutes (option 2)
- Adjustment at  $51^{\circ}\text{C}$  before starting (option 1)
- Temperature should rise up to  $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$  within 2 minutes after the beginning of the treatment
- Temperature should not go down under  $49^{\circ}\text{C}$  during the treatment (for the 3 sensors)



# OPERATING METHOD IN CANADA

## WHY IS HOT WATER TREATMENT (HWT) NOW ACCEPTED AS A VIABLE SOLUTION?

- Prior to 2005, French Nurseries had little commercial experience with HWT and were reluctant to endorse its use on exported plants.
- In 2006, based upon new research and protocols, the region of Burgundy began to insist on HWT treatment of vines.
  - Over one million plants were successfully treated in the Burgundy region with less than 3% mortality.

The table below summarizes the difference in the original HWT protocol and the New HWT Protocol.

PREVIOUS HWT PROTOCOL	NEW HWT PROTOCOL
• HWT - 50°C for <u>45 minutes</u>	• 24 hour acclimatization at room temperature pre and post HWT
• Cool water soak to cool down vines rapidly	• HWT - 50°C for <u>35 minutes</u>
• Return to cold storage or plant	• 24 hours at room temperature
	• Fungicide dip treatment
	• Aerated packaging
	• Return to cold storage up to 8 weeks at 1-2 °C
	• Soak vines in water minimum of 24 hours prior to planting
RESULTS - high mortality loss	RESULTS - low mortality, approximately 3%, similar to non-treated vines

# OPERATING METHOD IN CALIFORNIA

HWTprocess currently involves three steps.

1. Immersion during 5 minutes into a pre-heating tank at 30°C to warm up the canes.
2. Immersion during 5 minutes into the HW tank at 52.8°C (127°F) to kill vine mealybugs.
3. Immersion during 5 minutes into a cooling tank at +/- 23°C : used as a buffer to prevent any damage on the plant material.

Several publications report that these treatments can be done without negative impact on the material

Hot-water treatments also have the added effect of providing partial to complete control of pests such as: root knot nematodes, grape phylloxera, Pierce's disease (*Xylella fastidiosa*), *Phytophthora cinnamomi*, *Flavescence dorée*, and *Agrobacterium spp.*

*Foundation Plant Services FPS Grape Program  
Newsletter October 2004*



## **Technically possible to adopt 50°C-35' under certain conditions :**

- Treatment stations should be adapted to these new conditions
- Required effective duration : 35' à 50°C  $\pm$  1°C considering machine's characteristics, type of material (top-graft cuttings, rootstock cuttings or plants). 50°C  $\pm$  0,5°C technically impossible to guarantee !
- Necessity to verify the conditions to raise temperature after the beginning of the HWT (plan verifications ahead by accredited organization)
- Sensors and recorders standardized and verified (Ex: sensor PT100 for immersion with exactitude of  $\pm$  0,5°C maxi and resolution of 0,1°C)
- Recording frequency: every minutes
- Efficacy on Bois Noir to be verified for this protocol (not tested yet)

# HWT PROTOCOL MODIFICATIONS

And after these conclusions. What procedure?

FFPV requested the French Ministry of Agriculture for the modification of the HWT's protocol

The Ministry of Agriculture requested the opinion of ANSES about this proposal

ANSES carried out a study on the efficacy of HWT against grapevine yellows and other diseases or pests.

ANSES, within this framework, audited IFV about the efficacy of HWT against phytoplasma

Not yet official opinion of ANSES released but ... we have some indications through their presentation during the last seminar on *Xylella fastidiosa*...



French equivalent of EFSA  
(European Food Safety Authority)

Evaluation of methods to control *Xylella fastidiosa*

## Evaluation des méthodes de lutte contre *Xylella fastidiosa*



Charles Manceau  
Anses





# Seminar Xylella Fastidiosa 16 september 2016

## ANSES' intervention about HWT efficacy

Request by the Ministry for a ANSES's opinion about the HWT's protocol modification

**Demande d'avis relatif à la modification du couple temps-température du traitement à l'eau chaude du matériel végétal**

2015-SA-0265



COURRIER ARRIVÉ  
28 DEC. 2015  
DIRECTION GÉNÉRALE

MINISTÈRE DE L'AGRICULTURE, DE L'AGRO-ALIMENTAIRE ET DE LA FORÊT  
Direction générale de l'alimentation  
Service de la prévention des risques  
sanitaires de la production primaire  
Sous-direction de la qualité et de la  
protection des végétaux  
Bureau des hermines et de la Santé  
des Végétaux  
207, rue de Vaugrand  
75022 Paris cedex 13  
Cliquez sur le lien : [Séminaire CLANESC](#)  
Tél. : 01 49 23 28 24  
Mét. : [www.msa.gouv.fr](#)  
Site : [www.msa.gouv.fr](#)  
N° de dossier : 1555V-2015-12-007

Ministère de l'Agriculture, de l'Agro-alimentaire et de la Forêt  
Ministère de l'Environnement, de l'Énergie et du Climat  
Ministère de la Santé  
27-31 avenue du général Leclerc  
93147 La Courneuve cedex

Permis : 18 DEC. 2015

Objet : Saisine relative à la modification du couple temps-température du traitement à l'eau chaude du matériel végétal

- 1- Déterminer l'efficacité du traitement à l'eau chaude (TEC) pour la Flavescence dorée (FD) et *Xylella fastidiosa* (Xf) avec couple temps/température de 35 minutes à 50°
- 2- Déterminer l'efficacité du traitement à l'eau chaude avec les couples temps/température de 35 minutes à 50° et de 45 minutes à 50° sur les organismes suivants :

- jaunisses de manière générale
- *Xylophilus ampelinus*
- Phylloxera
- *Agrobacterium tumefaciens* et *Agrobacterium vitis*
- nématodes vecteurs de virus
- insectes et plus particulièrement aux stades de développement œufs, larves, nymphes.

HWT efficacy against different diseases and pests

# Seminar Xylella Fastidiosa 16 september 2016

## ANSES' intervention about HWT efficacy

1- Déterminer l'efficacité du traitement à l'eau chaude pour Flavescence dorée (FD) et *Xylella fastidiosa* (Xf) avec couple temps/température de **35 minutes** à 50° C.

- Saisine soumise suite au rapport technique de l'IFV

- Montrant qu'un traitement à 50° C pendant 35 min permettait d'assainir le matériel végétal de vigne vis-à-vis de FD

Essais réalisés à la demande de la FFPV et avec un cofinancement FAM – Marque Entav-Inra®



2015-SA-0266

TRAITEMENT A L'EAU  
CHAUDE DES BOIS ET  
PLANTS DE VIGNE

PROGRAMME FRANCEAGRIMER 2011-2012

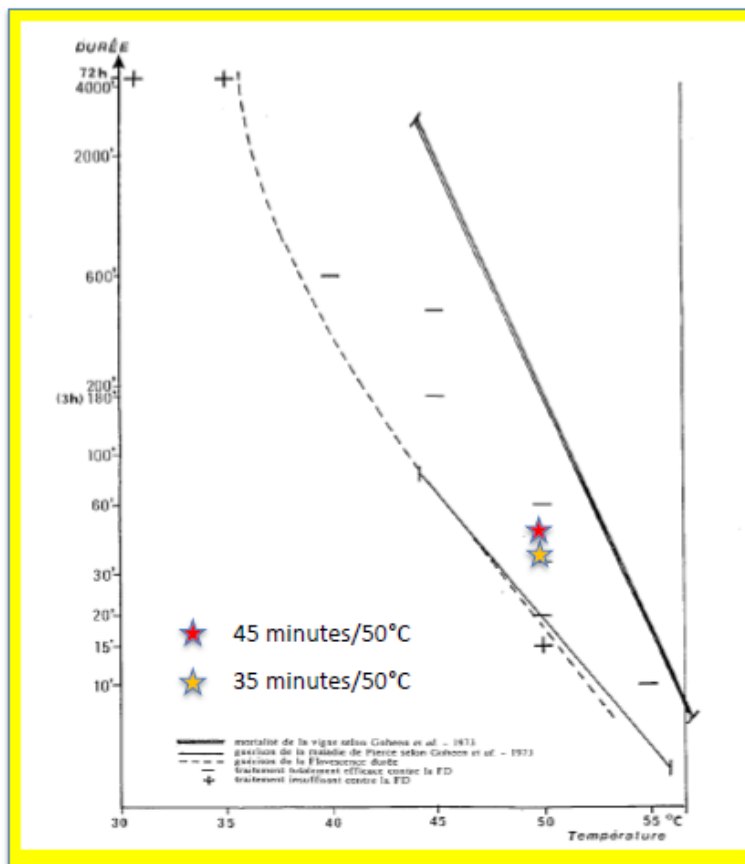


RAPPORT TECHNIQUE

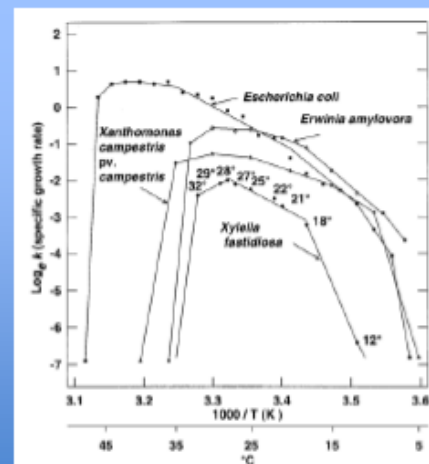
- Audition de M. P. Bloy (IFV)

Same bibliographic data as ours

### Analyse bibliographique



D'après Caudwell et al., 1990 qui intègre les résultats de l'INRA sur la FD et de Goheen et al., 1973 sur *X. fastidiosa*



Taux de croissance *in vitro* de *X. fastidiosa* à différentes températures (Feil & Purcell, 2001)

HWT efficacy against vine pests and diseases

ANSES's opinion

## Efficacité des TEC sur les organismes nuisibles à la vigne

Organismes nuisibles	Durée du traitement à 50°C			
	45 minutes		35 minutes	
	efficacité	incertitude	efficacité	incertitude
Flavescence dorée	+	0	+	0
Bois noir	+	0	-	0
Jaunisses	+	élevée	+	élevée
<i>X. fastidiosa</i>	+	0	+	0
<i>X. ampelinus</i>	+	faible	+	faible
<i>A. vitis</i>	+	modérée	+	modérée
<i>A. tumefaciens</i>	+	élevée	-	faible
Phylloxera	+	modérée	+	modérée
<i>Scaphoideus titanus</i> (œufs)	-	faible	-	faible
<i>Planococcus coccus</i>	+	modérée	+	modérée
Nématodes vecteurs de virus	+	faible	+	faible

Same conclusions as ours !

## Conclusions

- L'application de TEC sur des bois de vigne assainit de façon générale les organes traités vis-à-vis des bioagresseurs de la vigne. Cependant, les bioagresseurs ont des sensibilités différentes aux traitements.
- Les différentes données bibliographiques et les essais récents de l'IFV montrent qu'un traitement des bois et plants de vigne à l'eau chaude pendant **35 minutes à 50°** est efficace pour détruire le **phytoplasme de la FD**. Les données bibliographiques indiquent qu'il en est de même pour *X. fastidiosa*.
- L'abaissement de la durée du traitement de 45 à 35 minutes ne peut s'envisager que si la température de consigne du traitement de 50 ° est suffisamment maîtrisée pour que la température réelle ne descende jamais en-dessous de 49° durant 35 minutes.
- En outre, pour maintenir une durée de traitement réellement efficace, celle-ci doit être considérée quand le bain est à la température de consigne, c'est-à-dire après stabilisation de la température suite à l'immersion des plantes.

ANSES's official opinion expected by the end of the year



## Next step:

Official request to Europe by the French Ministry of Agriculture.

But it's necessary that the other countries agree this modification's proposal .

➡ So, finally, we hope you will support this proposal !

# HOT WATER TREATMENT



THANK YOU FOR  
YOUR ATTENTION