European Food Safety Authority

Mortality Verification of PWN from High Temperature Treatment of Shavings

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Scientific Assessment Support (SAS)

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Regulation

- Pinewood Nematode (PWN)
  (Bursaphelenchus xylophilus (Steiner and Buhrer) Nickle)
  is listed as harmful organism in PLH regulation
- Council directive 2000/29/EC Annex IV, Part A, Sec. 1, Point 1.2:
  Wood of conifers (except *Thuja* L.) in form of shavings originating in the US need a

  Heat treatment (56/30)
  - achieve a minimum core temperature of 56°C
  - for at least 30 minutes

- According to ISPM No.15 for wood package material

Request

In view of technical difficulties
on practical application
of the (56/30) treatment
USDA requested the recognition
of an alternative

(Photo from Jones, 2009)

Heat treatment (398/3) with continuous flow

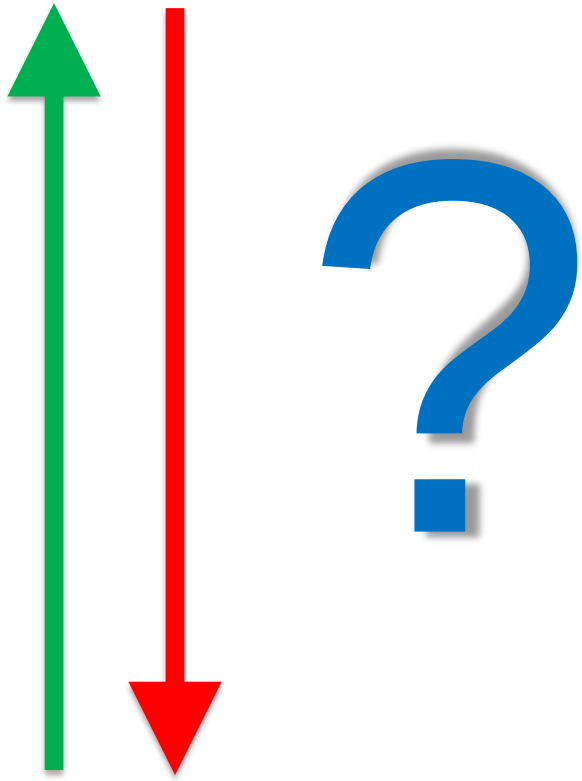
- exposing shavings to high temperature 398°C
- for 3 minutes

supported by a research experiment of
Jones J (2009):
Mortality Verification of PWN from High Temperature Treatment of Shavings

Reasoning

The shorter the time
the lower the mortality

The higher the temperature
the higher the mortality



Experimental Setting

16 white pine logs infested by PWN
Date (detection): 27th May 2008, Day 0

3/8 inch drill, 4 inch knife **445g shaving and slices** Date: 1st June 2008, Day 5		Shredder, mesh conveyor **9595g shaving and slices** < 3 mm
Devision **245g Control**	Devision **200g Test**	Heat treatment: **398°C / 3 Minutes**
Subsamples **4 × 10g**	Subsamples **4 × 10g**	Subsamples **30 × 5g**
Bearmann funnel 72h Date: 28th July = Day 62 Mean: **3.25 PWN / 10g**	Bearmann funnel 72h Date: 6th June = Day 10 Mean(±Std): **233.5 (±13.6) PWN/10g**	Bearmann funnel 72h Date: 16th June = Day 20 Mean(±Std.): **0 (±0) PWN / 5g**

Is this sufficient?

Structure of Evaluation

EFSA Guidance on Methodology for Evaluation of the Effectiveness of Options for Reducing the Risk of Introduction and Spread of Organisms harmful to Plant Health in the EU Territory

(EFSA Journal 2012, pp. 2755 [92p.])

Information requirements:

1. Description of the proposed risk reduction option (RRO)

2. Experimental assessment of the effectiveness under laboratory/controlled conditions

3. Analysis of the applicability of the risk reduction option

Description of RRO

Item	Description
Name	Hight temperature treatment of shavings (398/3)
Target pest	Pinewood Nematode (PWN) (*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle)
Target plant material	Coniferous wood (except *Thuja* L.) in regulation / *Pinus* spp. in request for derogation
Origin of plant material	United States (U.S.)
Type of RRO	Heat treatment: 398°C / 3 minutes
Place of implementation	United States (U.S.)

Experiment Assessment

Item	Description
Pest information: - Identity - Conditions infestation - Life stage / resistance	Pinewood nematode - No information on identification (versus *B. mucronatus, B. kolymensis, B. fraudulentus*) - Natural infestation - No information on distribution of life stages
Plant material: - Type / plant identity - Conditions	White pine (*Pinus strobus*) - 20 days after detection of the pest - Shaving and slices, control: from drill, treated: <3mm
Experiment: - Measure for effect - Factors controlled - Factors uncontrolled - Equipment - Treatment - Monitoring factors	Heat treatment - Counts of PWN in controls and after treatment - Air temperature in own, time - Type of wood, wood moisture, material density in own, homogenity of temperature in own, core temperature in wood - Dryer with a continuous material flow - Bearmann funnel
Statistics: - Design - Data / analysis - Conclusions	No observed alive PWN after treatment - 2 control groups (Day 5, 62), 1 treatment (Day 20) - Calculation of mean infestation

Conditions of Application

Item	Description
Implementation: - Place / equipment - Treated material - Indirect effects	Unknown
Monitoring: - Parameters - Critical thresholds - Equipment	Unknown

Comparison: Pest

Risk reduction option	Experiment
Pinewood Nematode (PWN) (*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle)	Pinewood nematode - No information on identification (versus *B. mucronatus, B. kolymensis, B. fraudulentus*) - Natural infestation - No information on distribution of life stages

- No controlled infestation in the experiment
  - No. PWN / 10g
  - Distribution of life stages, e.g. most resistant (Third dispersal juvenile stage J_{III})
- No (clear) identification of PWN

Comparison: Material / Hosts

Risk reduction option	Experiment
Coniferous wood (except *Thuja* L.) in regulation *Pinus* spp. in request for derogation	White pine (*Pinus strobus*) - 20 days after detection of the pest - Shaving and slices, - Control = drill, treated = shredder <3mm

- Only White pine in the experiment
- No controlled conditions of storage
  - Natural mortality

Comparison: Material / Product

Risk reduction option	Experiment
Coniferous wood (except *Thuja* L.) in regulation *Pinus* spp. in request for derogation	White pine (*Pinus strobus*) - 20 days after detection of the pest - Shaving and slices, - Control = drill, treated = shredder <3mm

- Different production of shavings
  - Control 3/8 inch drill
  - Treated shredder with mesh conveyor

- Effect of production and treatment not distinguishable

Influencing Factors

- Type of wood
- Wood moisture
- Air temperature in own
- Material density in own
- Homogenity of temperature in own and during the whole treatment
- Core temperature in wood
- Time

Comparison: Treatment

Risk reduction option	Experiment
Heat treatment: 398°C / 3 minutes	Dryer with a continuous material flow: - air temperatur 398°C - 3 passes (e.g. 3 minutes)

- Temperature at start unclear,
  due to loading of shavings with ambient temperature
- Temperature of shavings after third pass reported as 115°C

- Temperature of 398°C close to charring (from 380°C)

- Core temperature of 398°C during 3 minutes not assured

- No information on homogenity of the process

Control of Influencing Factors

- Only one combination
  of temperature and duration (398/3) tested
- No control on infestation
- No control on wood conditions
- Different production methods of shavings

Statistical Evaluation: Principles

- Uncertainty about the RRO should be reported
  - e.g. by confidence intervals etc.
- Probit 9 (mortality rate=99.9968%) should be used as treshold
- Statistical principles should be used for verification
  - e.g. power of tests

Statistical Evaluation: Timing

Control	Test	Treatment
Day 62 Mean: **3.25 PWN / 10g**	Day 10 Mean(±Std): **233.5 (±13.6) PWN/10g**	Day 20 Mean(±Std.): **0 (±0) PWN / 5g**

$$E(N_t) = E(N_{10}) \cdot \quad \exp(-\lambda \cdot (t - 10))$$

- Mean infestation (day 10): 233.5 PWN/10g
  - 95% confidence interval: [190.2, 276.8] (Normal); [218.8, 248.8] (Poisson)
- Survival rate per day: 92.1%, CI: [91.8%, 92.5%]
- Mean infestation (day 20): 51.3 PWN/5g
  - 95% confidence interval: [49.7, 53.4]

Model for Timing

$$E(N_t) = E(N_{10}) \cdot \quad \exp(-\lambda \cdot (t - 10))/2$$

Exponential decrease
with

E(N_{10}) = Expected number of nematodes per 10g at day 10 (June 6)
-λ = logarithmic survival rate over time
s = exp(-λ)survival rate over time
E(N_t) = Expected number of nematode per 5g at day *t*.

Statistical Evaluation: Effect

The results show that:

- when the mortality rate is 99.85%,
  there is 10% chance (power 90%)
  to observe zero nematodes alive in 30 samples,
- when the mortality rate is 99.81%,
  there is 5% chance (power 95%)
  to observe zero nematodes alive in 30 samples,
- when the mortality rate is 99.7%,
  there is 1% chance (power 99%)
  to observe zero nematodes.

Model for Effect

$$y_t \approx Poisson[E(N_t)]$$

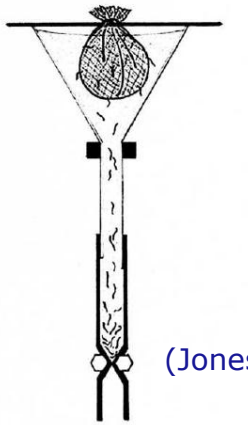
$$E(N_t) = E(N_{10}) \cdot \quad \exp(-\lambda \cdot (t-10)) \quad \cdot (1-h)/2$$

Observation is Poisson distributed with expected value $E(N_t)$
and

$E(N_{10})$ = Expected number of nematodes per 10g at day 10 (June 6)
$-\lambda$ = logarithmic survival rate over time
s = exp(-λ)survival rate over time
h = Mortality rate of the "Heat treatment"
$E(N_t)$ = Expected number of nematode per 5g at day *t*.

Detection of Pest: Method

As detection method
a Bearmann Funnel was used
with 72h extraction:



(Jones, 2009)

- Failures in recover nematodes reported .
  (Evans et al. 1996)
- Modifications of method not stated in the experiment.

…a bit Statistics: How Many Insects?

Sample size needed to confirm different mortality rates by “no survivors” (with significance level α=5%)

Significance	α =0.05		
	Survival	Mortality	Sample size
probit	π	q=1-π	n
	10.0000000%	90.0000000%	29
	1.0000000%	99.0000000%	299
	0.1000000%	99.9000000%	2995
	0.0100000%	99.9900000%	29956
	0.0010000%	99.9990000%	299572
	0.0001000%	99.9999000%	2995731
1	15.8655254%	84.1344746%	18
2	2.2750132%	97.7249868%	131
3	0.1349898%	99.8650102%	2218
Probit9 = 4	0.0031671%	99.9968329%	94587
5	0.0000287%	99.9999713%	10450778

…a bit Statistics: How Many Samples?

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Probability to find at least one of 10 infested trees in a population of 8000

r	K	n	N	P(X=0)	P(X>=1)
5%	400	10	8000	59.86%	40.1%
10%	800	10	8000	34.85%	65.2%
15%	1200	10	8000	19.67%	80.3%
20%	1600	10	8000	10.72%	89.3%
25%	2000	10	8000	5.62%	94.4%
30%	2400	10	8000	2.82%	97.2%
35%	2800	10	8000	1.34%	98.7%
40%	3200	10	8000	0.60%	99.4%
45%	3600	10	8000	0.25%	99.7%
50%	4000	10	8000	0.10%	99.9%
55%	4400	10	8000	0.03%	100.0%
60%	4800	10	8000	0.01%	100.0%
65%	5200	10	8000	0.00%	100.0%

Comparison: Application

Risk reduction option	Application
Only specified: 398°C / 3 minutes	Implementation: Unknown - Place / equipment - Treated material - Indirect effects
Not specified:	Monitoring: Unknown - Parameters - Critical thresholds - Equipment

The conditions of application were not specified.

It must be guaranteed that the treated shavings are isolated from the other processing (mesh conveyor).

Final Opinion

- The temperature of the wood shavings in the experiment is unknown..
- A number of factors that may influence the results of the experiment are unknown.
- No information is provided to confirm the identity and the frequency of occurrence of the most resistant life stages of B. *xylophilus* in the treated samples.

The panel agrees that there is a potential for development of an alternative treatment protocol based on exposure of wood shavings to high temperature for a short duration. The panel however, does not consider that the evidence presented in the report demonstrates the effectiveness and reliability of the proposed temperature time regime.
(EFSA 2009)

Thank you for your attention

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