FPInnovations

Evaluation criteria for phytosanitary treatments of wood packaging

Alternative approach to probit 9

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Introduction

- Many species of insects, nematodes and fungi may colonize wood especially recently dead or dying trees
- Such lower quality wood, used as wood packaging is recognized as high-risk pathway
- ISPM15 adopted in 2002 to address the threat
  - HT (56/30) and fumigation with methyl bromide
  - Alternative treatments needed (HT not always available, MeBr to be phased out)






Introduction

- TPPT requested in 2007 that TPFQ develops evaluation criteria for new treatments for ISPM-15, include list of test pests and required efficacy (“probit 9” considered as it has been arbitrary used as a standard in fruit flies quarantine research)
- TPFQ asked International Forestry Quarantine Research Group (IFQRG) comprising of scientists, regulators, the industry representatives for help
- IFQRG worked on and discussed evaluation criteria over several years

Introduction

- ISPM No 28 – Phytosanitary treatments for regulated pests, does not specify required efficacy
- ISPM No 15 (mentioned other possible treatments but provided no specific guidelines on efficacy data)

Introduction

- In 2010 a draft appendix "Submission of new treatments for inclusion in ISPM 15" contained evaluation criteria for new treatments and was released for countries consultation
  - Included extensive list of target pests (including quarantine pests) and proscribed probit 9 as a required level of efficacy
  - Criticised as too complex and too stringent
- IFQRG reviewed the SC comments and worked on alternative approach especially to address the list of pests, and probit 9

Key points

- Alternative treatments for wood packaging are urgently needed
- Currently used methods (HT schedule adopted based on efficacy data for PWN and its vectors; Methyl Bromide has historically been accepted for variety of pests) are successfully used but did not go through that rigorous process
- Wording in ISPM15 2009 changed to" practically eliminate" to significantly reduce risk of introduction and spread. Zero risk=zero trade

Key points

- Probit 9 requirement and extensive list of pest including a quarantinable pests (ALB, EAB) is serious impediment and prevents new treatment development and adoption
- Initially there were many discussions, approaches, confusion, requests and disagreements but during the process of negotiation, education and increased understanding, IFQRG in principal agreed on alternative approach in Sept 2011. Two peer-reviewed papers published.

Haack et al, 2011. Seeking alternatives to probit 9 when developing treatments for wood packaging materials under ISPM No. 15 EPPO bulletin 41:39-45

Schortemeyer et al 2011. Appropriateness of Probit-9 in the Development of Quarantine Treatments for Timber and Timber Commodities J. Econ. Entomology, 104(3):717-731)

Wood packaging is a specific commodity

- Could harbor many and diverse pests that can vary between countries and WPM

- WPM go around the world repeatedly
- Treatments (measures) must target multiple pests instead of discrete species or genus

Insects

–Bark-inhabiting beetles (6,000 Scolitinae)
–Wood-inhabiting beetles (20,000 Cerambicids)
–Adelgids and scales
–Termites, Carpenter ants
–Wood-inhabiting moths
–Bark-inhabiting moths
–Wood wasps

Fungi

–Rust fungi
–Decay fungi
–Canker fungi
–Bluestain fungi
–Black yeasts
–Vascular wilt fungi
–Oomycetes

Others

Mistletoes
Nematodes (e.g. PWN)
Mites,
Viruses,
Bacteria

How to test efficacy of a treatment?

- Set up experiment, chose specimens and expose them to a treatment
  - Which organisms (stages) and how many do you need to test to represent all possible pests around the world
  - Do you test specimen alone or infested wood as one unit
  - Can you use naturally infested wood or lab inoculated,
  - Lab based tests versus scaled up field tests
  - What replication is needed; what is acceptable statistcs
  - How to address the worse case scenarios: e.g most difficult to treat substrate type, effect of moisture content, frozen wood, with/without bark
  - What if some organisms survive the treatment, what is acceptable

A Question of numbers/replication

- If you use 10 insects (or 10 infested log units) and manage to kill all of them using a particular treatment, does that make a 100 % efficacy and can can you submit such data?

“Efficacy” versus “Reliability”

- Efficacy (common word): The ability to produce a desired amount of an effect.
- Reliability (statistical): The effectiveness of the treatment at a given confidence intervals; expressed as decimal portion of 1.0
    - Probability that the same result will be obtained again and again with repetition.
    - It addresses natural tolerance variability within test pest. A sample needs to represent the whole population

Common statistical formula for sample size and statistical reliability

Sample size necessary to demonstrate a given reliability at 95% confidence level :

$n=\log(0.05)/\log(p^r)$

Statistical reliability: probability that the same result will be obtained again and again with repetition

n	reliability
1	0.050000000
5	0.549280272
10	0.741134449
15	0.818963727
20	0.860891659
25	0.887071855
30	0.904966147
35	0.917968364
40	0.927842475
45	0.935595711
50	0.941844921
59	0.95049239
60	0.951297087
100	0.97048695
299	0.990030853
2995	0.999000256
93613	0.999968

Two approaches to test reliability

- **BRUTE FORCE EXPERIMENTS - Involves exposing n number of units to the selected dose and no units should have any survivors**
  - 0.95% effective n=59
  - 0.99% effective n=299
  - 0.999% effective n=2995
  - **0.999968% (Probit 9)   (95% confidence) n=93,613**

- **DEVELOP MORTALITY CURVES – Dose Response Models**
  - Develop a mortality curve (probability of death as a function of temperature/exposure time that could be confidently used to determine the efficacy of any choice of thermal death time.
  - Done through extrapolation, so you need to accurately model the curve to achieve minimal variability within data – controlled lab experiments needed

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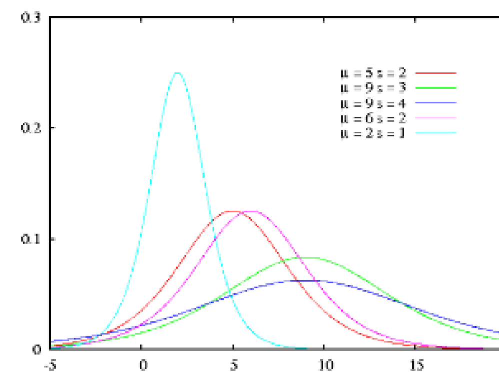
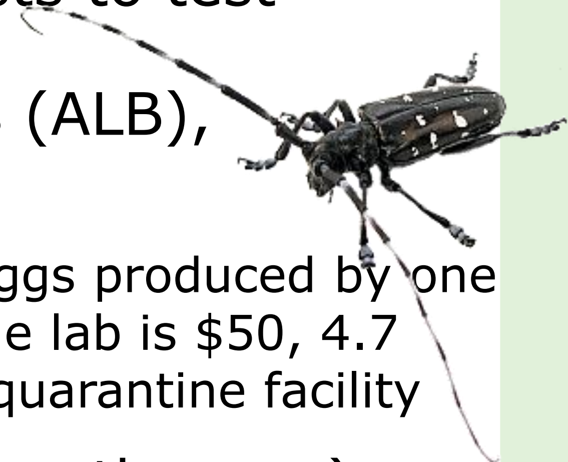


This has been done with fruit flies

- Easy to rear in the lab, easy to put in a jar or collect fruits that are heavily infested, to treat and get probit 9 reliability. But what about wood pests?

Not easy with wood pests

- There are potentially large diversity of pests to test
- Scarce availability of key quarantine pests (ALB), impossible to find 93,613 individuals,
  - Rearing takes 6 months to over year, only 50 eggs produced by one female in lifetime; cost of rearing one ALB in the lab is $50, 4.7 million to rear and treat 93,613 individuals, in quarantine facility



- Lack of separate units in some pests (e.g. pathogens)

- Results from modeling give overestimated values

What is a Reasonable Target for reliability (efficacy)?

The short answer:

Whatever the trading partners and their scientific advisors are comfortable with, should be based on pest-risk assessment.

Countries and their advisors do not necessarily agree on this

Probit 9 - key criticism

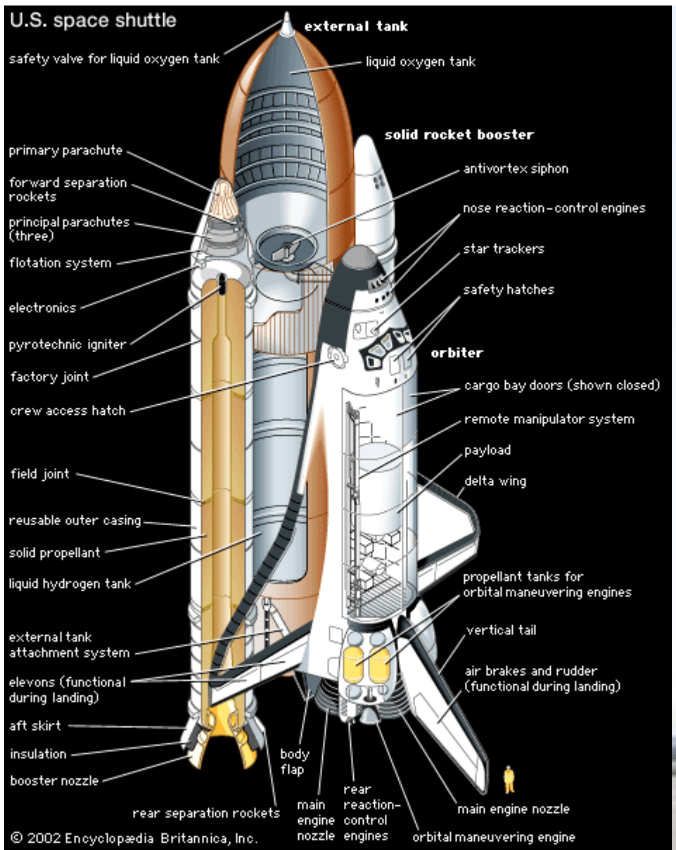
- Probit 9- based on work with fruit flies; wood products and pests are different
- There was questionable scientific reasoning to justify its use
- Many tests have to be done through brute force method (to kill 93, 613 individuals) but scarce availability of key quarantine pests (ALB), makes tests impossible to conduct
- Results obtained through modeling give overestimated values
- Large diversity of wood pests that need to be tested increases cost
- Lack of separate units in some pest (fungi) represent challenge

What shall we do?

How to develop an acceptable approach and trust it enough to allow fast development and adoption of new treatments and support safe trade?

Quality systems used by structural engineers – Phase 1, 2 and 3

- Used successfully for centuries to adopt a new component/structure (e.g. house, airplane, nuclear power plant, space shuttle….)



Phase 1

- A committee of professional engineers identifies the most critical types of failures.
- Proponents of new components asked to submit a engineering test results based on small lab testing

Phase 2

- New component with promising small scale test results is required to be tested in SCALED –UP EXPERIMENT to demonstrate higher reliability.
    - The scale depends on intended application and consequences of failure. At this stage testing can be cost prohibitive or impossible (e.g. new space shuttle component-no way to replicate in-situ conditions)
- However the engineers and the engineering committee are willing to come up with a test and accept risk and engineering process and progress is not stopped just because there are risks
- Successful large scale test results lead to PROVISIONAL APPROVAL of the component

Phase 3

- Structures that suffer failure are subject to POST FAILURE EXAMINATION to determine the cause

- If it is found that the new component was the cause-then it is BACK TO DRAWING BOARD.

- The component is restricted for use in any more structures that could encounter that type of failure in service until the proponents demonstrate that can be used safely, Re-design the component, or redesign the structure

IFQRG’s had similar 3-Step approach

- Step 1:Small lab scale test-estimate the lethal dose of the most tolerant pest of quarantine importance that is found in wood packaging
- Step 2:Larger lab test- replicate experiments at the estimated lethal dose for the most tolerant pest to demonstrate higher reliability
- Step 3: Simulated field test- confirm under simulated operational condition and provide statistical efficacy

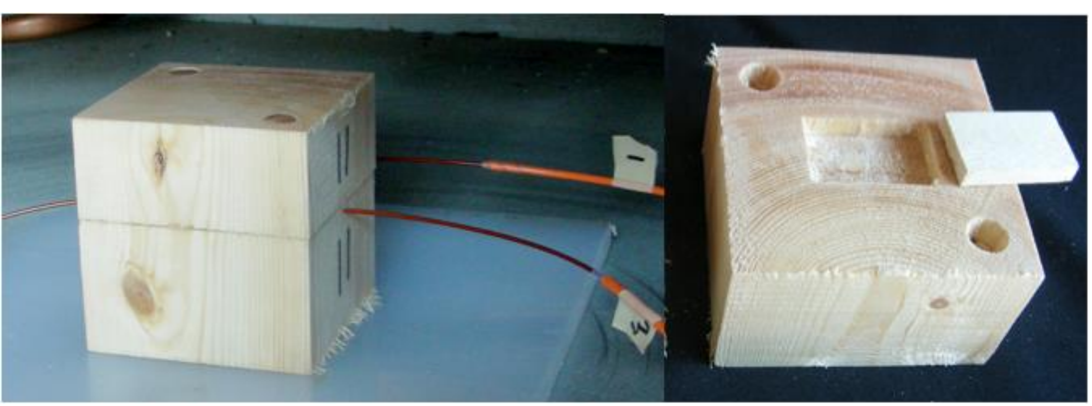
Approved treatment will be scrutinized on ongoing base in subsequent real-life experience, and either kept, modified or withdrawn (e.g. discussions on HT tolerance of EAB)

Step 1 (lab): Estimating Lethal Dose of Most Tolerant Life Stage

- Estimate treatment dose at which nearly all tested individuals will die using small sample size (5-10 experimental units each that can contain one or more target pests)

- Use smallest wood samples as test units to ensure uniform dose delivery

- How to address huge variety of possible pest and quarantine pest?



Step 1 (adjusted at IFQRG 2011)

- Pre-screening for tolerance (low replication). Test one available species from 7 representative pest groups:
  - a reference-easy-to-rear insect (e.g. from *Sitophilus, Oryzaephillus, Trogoderma* or *Ambrosia* beetle genera),
  - Scolytinae (bark beetles)
  - Bostrychidae (horned powder post beetles)
  - Buprestidae (metallic wood boring beetles)
  - Cerambycide (large wood borers)
  - Pine wood nematode,
  - Decay fungus from *Heterobasidion* genus,

  Other pests from the original list were dropped, after justifications were discussed (e.g. relevance to wood products and significantly reduced risk of pathway via wood packaging materials (e.g. Anobiidae, Lepidoptera: (Cossoid-Sessoid-Tortricoid assemblages, Siricidae, Fusarium circinatum, tree killing Phytophthora spp., deep penetrating blue stain fungi, canker fungi/chestnut blight, root rot fungi)

Step 2 (lab)

- Replicated experiments (with no survivors) at the estimated lethal dose using the most tolerant pests determined during step 1.
- Minimum sample size of 60 experimental units, which achieves 0.95 statistical reliability at the 95% confidence level.
- If possible test one or two doses above or below the estimated lethal dose
- If there are survivors , increase dosage until no survivors

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Note: Scientifically and statistically this gives better confidence in the results then treating 93,613 individuals in a single unit (pseudoreplication, as in work with fruit flies). Although it "meets" probit nine requirement (killing 93,613 flies) this is really a reliability of only 0.05 (sample size =1) and not reliability of 0.999968

Step 3 - Confirmatory Study Under Simulated Operational Conditions

- Using the most tolerant pests under simulated operational conditions using wood samples similar in size to wood packaging material and infested to levels that reflect field conditions. Sample size can be discussed and perhaps be 60 (0.95) to 300 (0.99).
- Pre test pest load can be estimated and used to calculate reliability figures following the treatment. When a sufficient amount of a pest is available (e.g. PWN) then the Probit 9 level will be easy to achieve and report but for more scarce pests the Probit level will be much less.

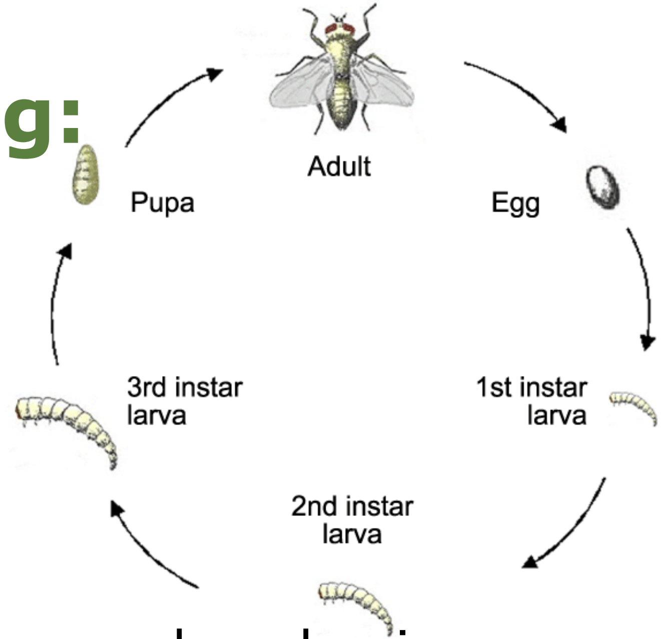
Why are there surviviors under operational conditions?

- Most like because the treatment was not delivered uniformly throughout the commodity and this needs to be addressed in parallel by treatment developers (e.g. Dialectric heating to be uniformly delivered throughout the profile of the wood)
- Understanding why some pests survived under operational conditions and how to proceed requires consideration of the whole system and pathway analysis?

“The Cardiff Protocol” IFQRG meeting in 2011 in Cardiff

The ‘Cardiff Protocol’ estimates a treatment efficacy target that reflects the biology of the pest, the pest relationship with wood packaging, and the trading patterns of wood packaging internationally.

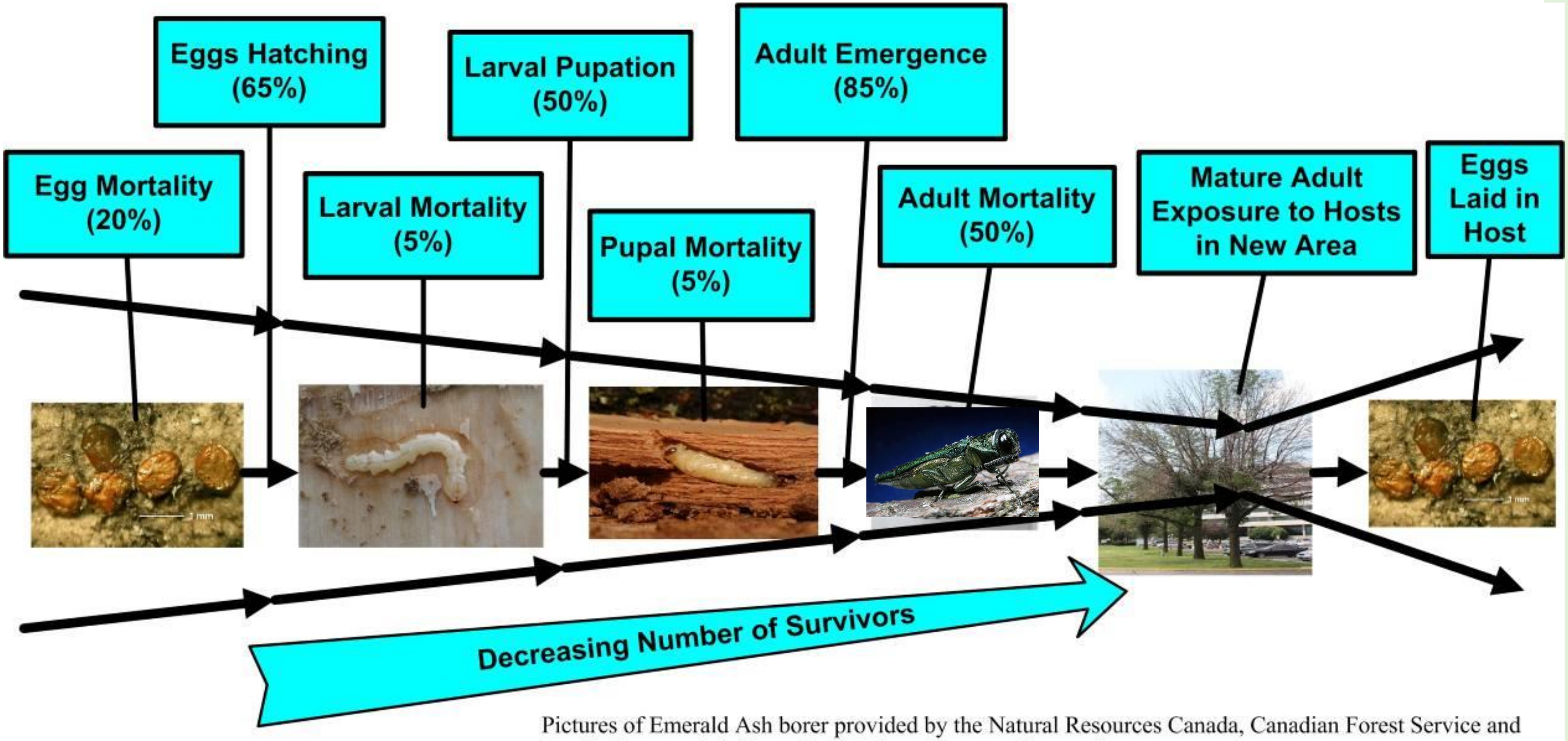
“Cardiff Protocol” model takes into account following:



- The biology of the pest
- The relationship between the pest and the wood packaging
- The international trading patterns of wood packaging material

"Cardiff Protocol" – biology of pest

- The level of natural mortality of the pest from the time of treatment to reproductive fertility



Pictures of Emerald Ash borer provided by the Natural Resources Canada, Canadian Forest Service and David Cappaert, Michigan State University, www.forestryimages.org

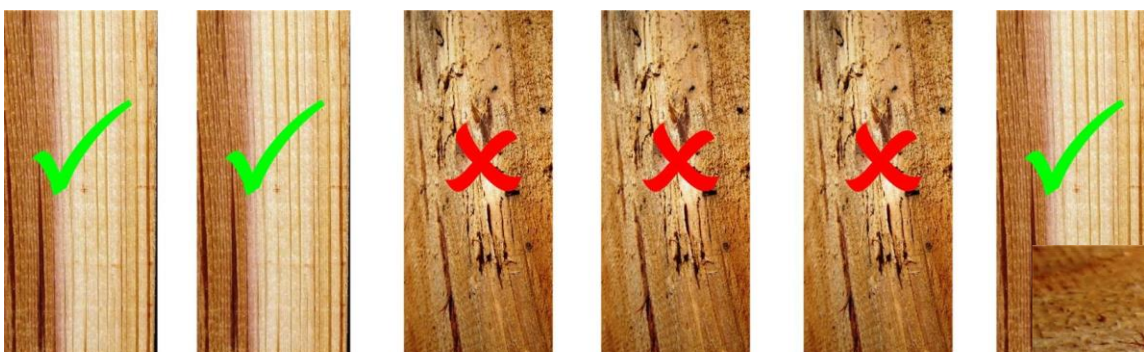
“Cardiff Protocol” –biology of pests

The founder population size (The number of individuals of the pest required to establish a new population) and level of natural mortality are combined to give the ‘**Maximum Pest Limit**’.

The **Maximum Pest Limit** or MPL is therefore the number of a pest infesting a unit of WPM that would be required to establish a new population in a new area.

"Cardiff Protocol"-relationship between pest and wood packaging

- The maximum ratio of pallets likely to be infested by the pest.
- The average number of the pest likely to be found in a unit of wood packaging.



“Cardiff Protocol” – trading patterns of wood packaging

- The maximum number of wood packaging units (e.g. pallets) found stacked or grouped together at a single location (e.g. a pallet recycling facility).

"Cardiff Protocol" – Required Pest Mortality

$$\frac{\text{Infestation Ratio} \times \text{Infestation Rate} \times \text{Number of Aggregated Units of WPM}}{\text{Maximum Pest Limit}}$$

”Cardiff Protocol” example for Southern pine beetle, *Dendroctonus frontalis*

- The **infestation ratio** = 75% (of pallets may be infested)
- The **infestation rate** = 60 larvae per pallet
- The **maximum Pest Limit** = 25 larvae (a Founder Population Size of 5 multiplied by a survival rate of 20%)
- The **maximum number of units** of WPM aggregated at a single location = 1000

‘”Cardiff Protocol” required pest mortality for *Dendroctonus frontalis*

$$\frac{\text{Infestation Ratio} \times \text{Infestation Rate} \times \text{Number of Aggregated Units of WPM}}{\text{Maximum Pest Limit}}$$

= 0.75 (75%) x 60 x 1000 = 45,000 larvae

= 45,000 larvae ÷ 25 (MPL) = **1,800**

This would require an experimental test size of only 5,400 larvae rather than 94,000 to provide acceptable efficacy data that will significantly reduce the pest risk

Concluding remarks

- Raising bar too will preclude new treatments to emerge
- Reliability levels need to be agreed and understood among those proscribing and accepting evaluation criteria for new tests
- Trading countries need to be willing to accept proposed test by a forum of knowledgeable individuals to manage risk. Forums such IFQRG provides a hub to secure an international agreement
- Understanding of pathway analysis is important in order to comfortably accept agreed reliability

IFQRG, TPFQ and TPPT are very close to agreeing on the final procedures

- Proscribed efficacy tests will be possible to do without bearing huge cost
- It will be scientifically sound and agreed among international group of scientists and regulators
- It will give confidence that treatments adopted and its guidelines will "Significantly reduce the risk of introduction and spread of pests"
- Following the adoption of new treatments real life experience will confirm the success and/or allow adjustments
- Treatments are just one measure as part of integrated approaches for safer and least restricted trade


Thank You

Extra slides

Case Study–Pine Wood Nematode (basis for HT schedule of 56/30min)

- Forintek (FPInnovations) was commissioned by the Canadian Federal Goverment to head a cooperative research program to examine Heat Treatment as alternative to Kilnd Drying for the eradication of Pine Wood Nematodes and its vector in green lumber

Guidance Provided to the Statisticians Designing the Experiments in 1991

- Canadian and EU authorities wanted to quantify the overall reliability (efficacy) of new plant health measures proposed to mitigate the risk that live PWN and its vectors could be present in shipments of Canadian lumber entering Europe.

- There were disagreements on number of points

Guidance Provided to the Statisticians Designing the Experiments in 1991

- A target reliability (efficacy) was not provided to the statisticians – and, not expected as disagreements on the target relaibility existed then as it exists now
- The statisticians assumed it would be extremely high (e.g. 0.99….) because of the massive volume of softwood lumber trade between Canada and the EU in 1991
- Statisticians also thought that the efficacy required to continue trade could change with improved data on the incidence of PWN in Canadian lumber or new knowledge
- Brute force method would not work so the modeling approach was taken

Approach

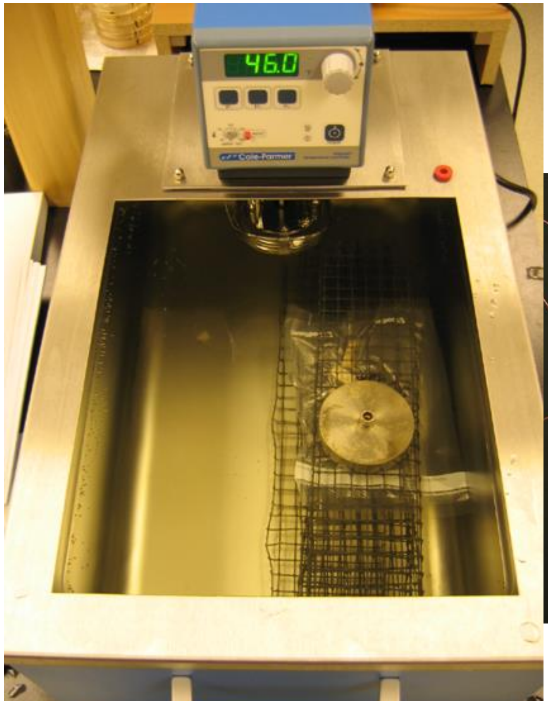
- A series of preliminary experiments were undertaken to identify:
  - most heat resistant Canadian softwood species
  - most resistant PWN isolates
  - worst-case wood moisture content for heat treatment of PWN in wood
- The results were used to design an experiment

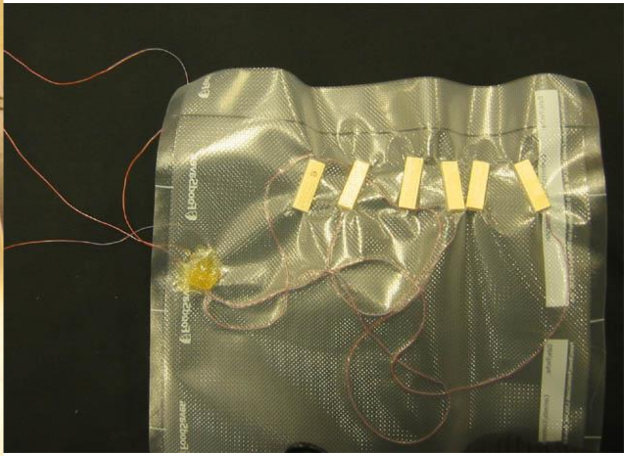
Method:

Experimental units:

- Small blocks of wood, inoculated with approximately 1000 nematodes each (to mimic the coolest part of any size piece of lumber containing PWN during industrial heat treatment).

Location: Laboratory - to control and precisely measure the temperature (dose) actually transferred to the nematodes in the blocks





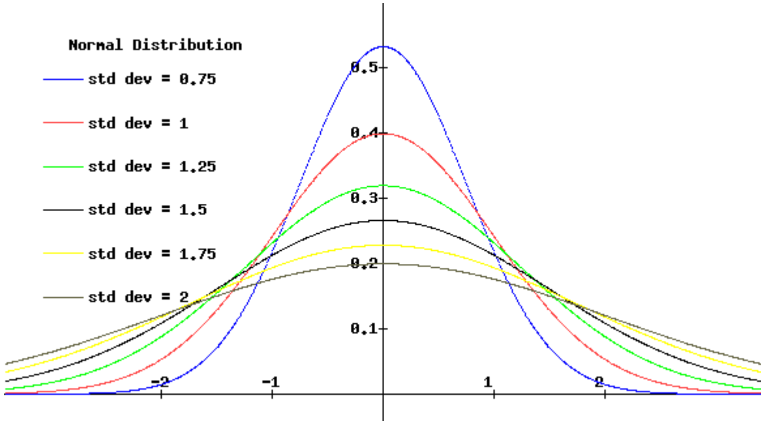
Results

Temper. °C	Time (min)	Number of Blocks	Number of blocks with 100% dead PWN
25	30	**60**	**1**
35	30	**60**	**6**
40	30	**60**	**16**
45	30	**60**	**34**
50	30	**60**	**59**
52	30	**59**	**59**
54	30	**60**	**60**
56	30	**60**	**60**
60	30	**60**	**60**
62	30	**60**	**60**
64	30	**60**	**60**
66	30	**60**	**60**

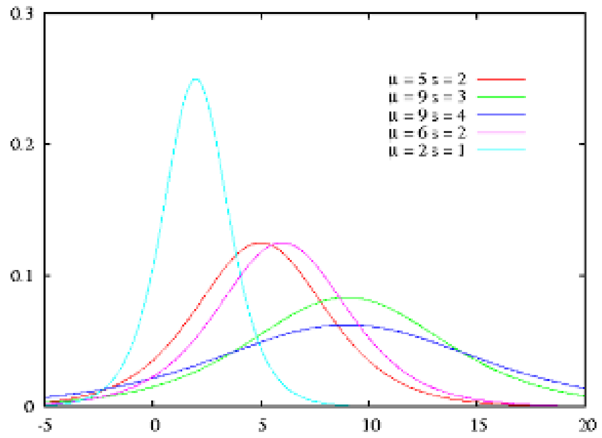
Follow probit analysis (Finney, D.J. 1971). Fit your data to a dose response models and do goodness fit tests to help you chose the best model

Three distributions

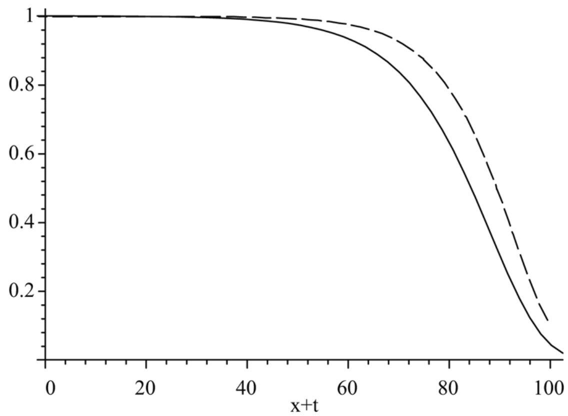
- NORMAL



- LOGISTIC



- GOMPERTZ

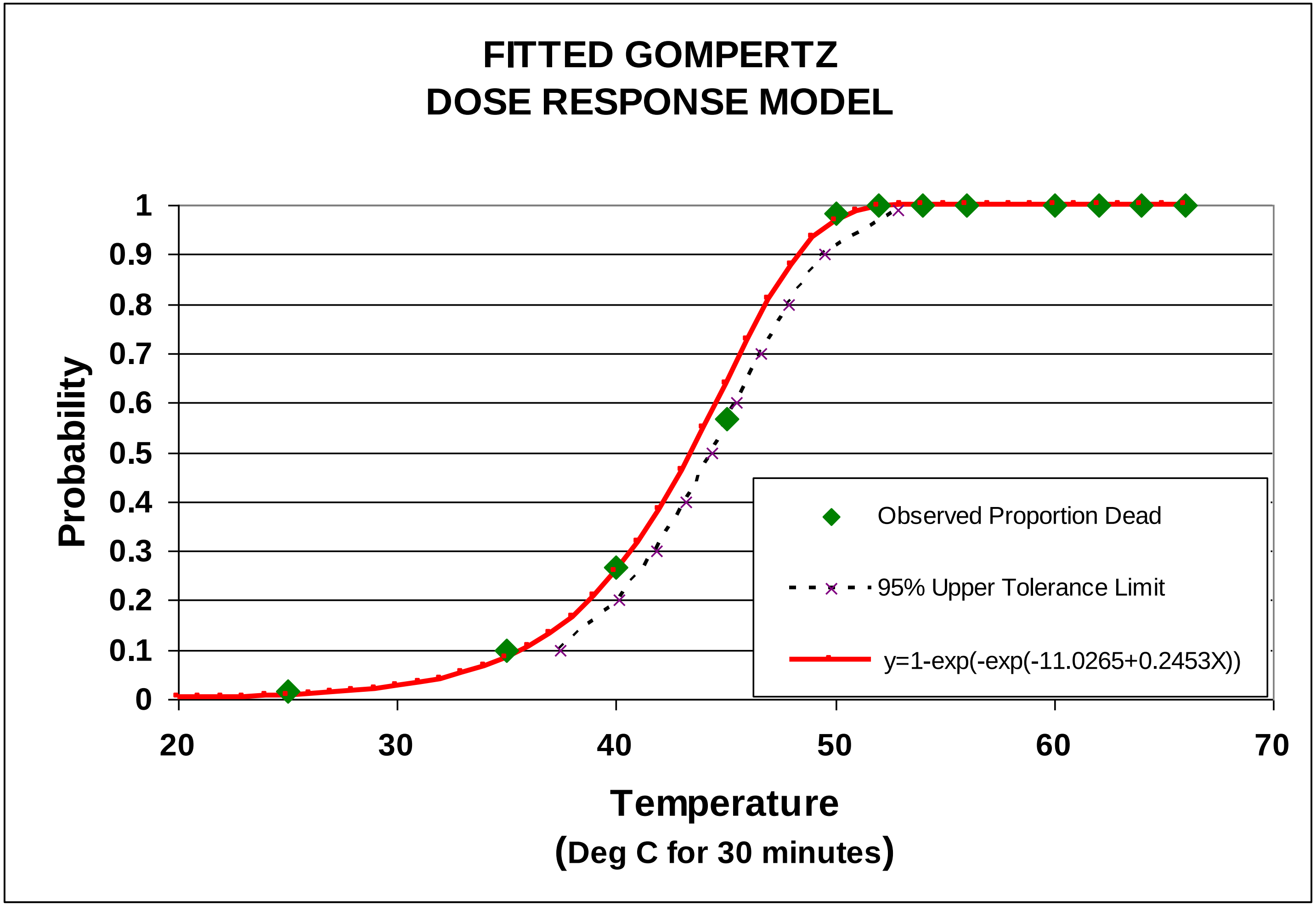


- Gompertz Benjamin (1779-1865) showed that the mortality is a geometric progression. When death rates are plotted on a logaritmic scale a straight line is obtained (Google Gompertz’s 1825 Law of Mortality)

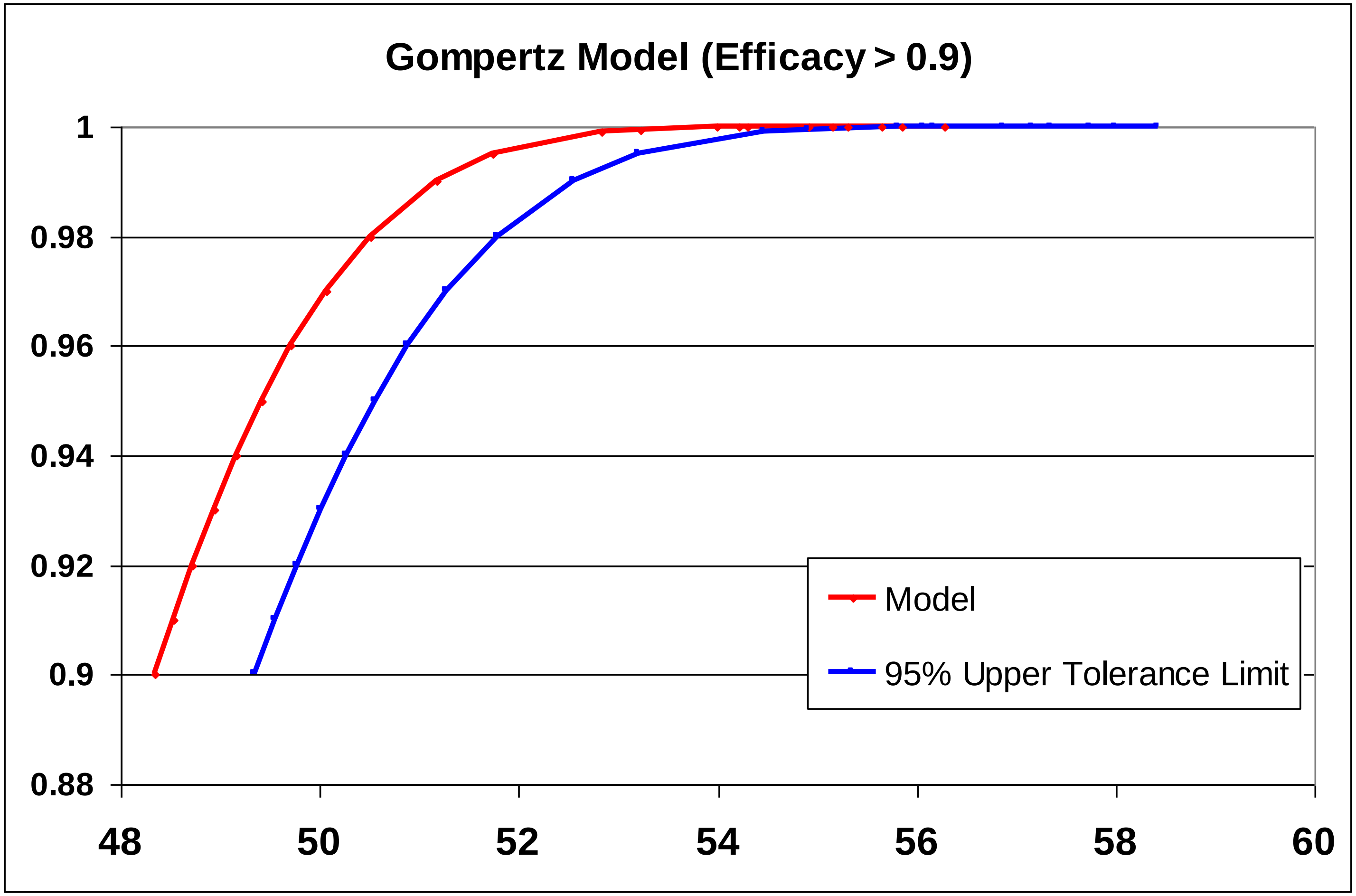
Results

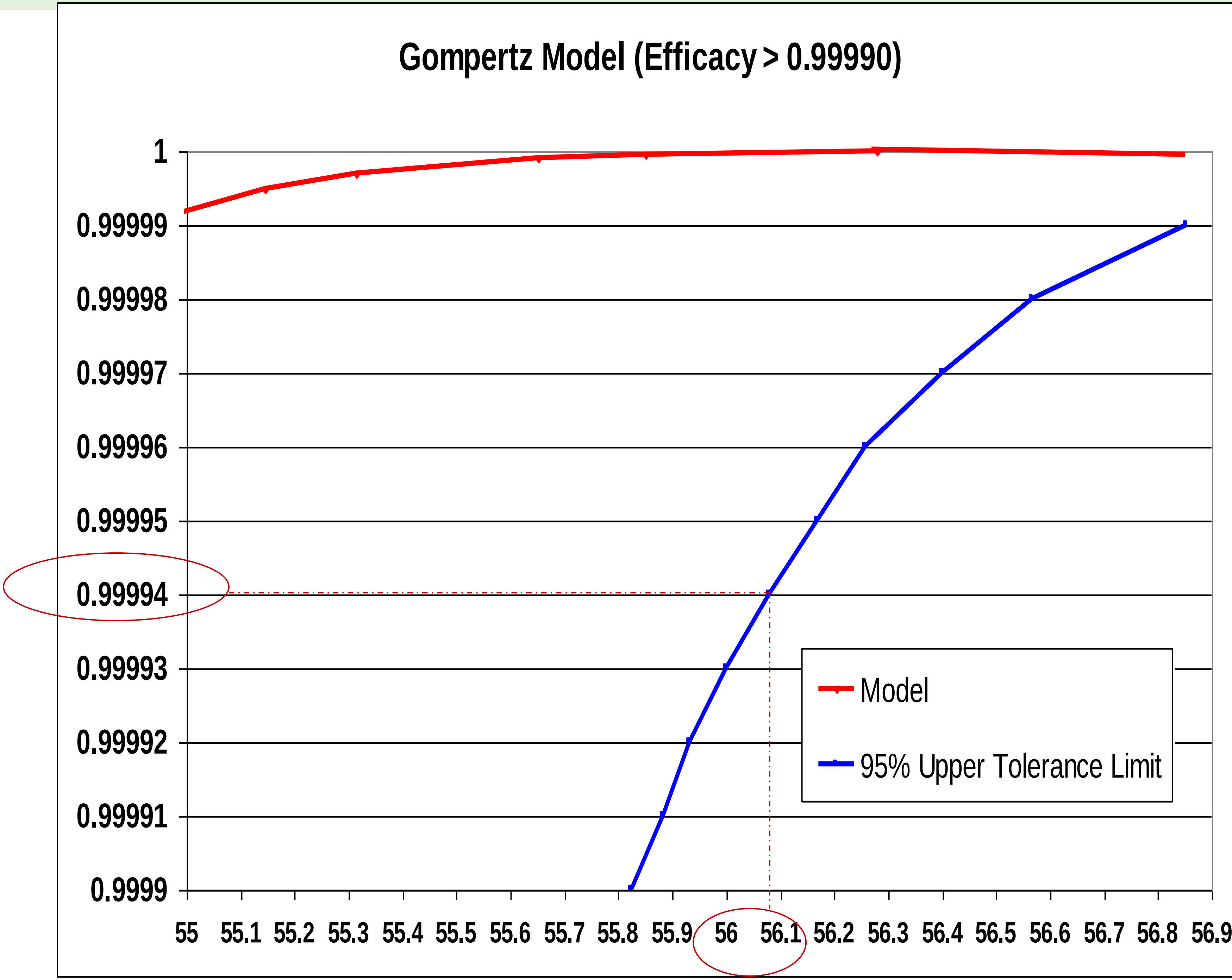
Temperature	Number of Blocks	Number of blocks with 100% dead PWN	Proportion (p) of blocks with 100% dead PWN	Mortality Curve
25	60	**1**	**0.017**	**0.007**
35	60	**6**	**0.1**	**0.083**
40	60	**16**	**0.267**	**0.257**
45	60	**34**	**0.567**	**0.637**
50	60	**59**	**0.983**	**0.968**
52	59	**59**	**1**	**0.996**
54	60	**60**	**1**	**>0.999**
56	60	**60**	**1**	**>0.999**
60	60	**60**	**1**	**>0.999**
62	60	**60**	**1**	**>0.999**
64	60	**60**	**1**	**>0.999**
66	60	**60**	**1**	**>0.999**

Mortality Curve - $ 5million -curve)



Blown up part of the Curve Where Efficacy > 0.9




Gompertz Model (Efficacy > 0.99990)
1
0.99999
0.99998
0.99997
0.99996
0.99995
0.99994
0.99993
0.99992
0.99991
0.9999
55
55.1
55.2
55.3
55.4
55.5
55.6
55.7
55.8
55.9
56
56.1
56.2
56.3
56.4
56.5
56.6
56.7
56.8
56.9
Model
95% Upper Tolerance Limit

Pathogenic fungi, commodities, treatments

- Phytosanitary treatments combined with other integrated measures may significantly reduce fungal ability to spread and establish, even though some fungi survived the treatment
- Pathogenic fungi may be present in a commodity (excluding P4P) but may have reduced risk of establishment e.g. they lack of competiveness, or ability to produce spores
- Their detection does not necessarily mean TROUBLE.