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Creating forest sector solutions

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Proving that a pest is eradicated or cannot spread out of the wood

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Traded Wood – Different Degree of Risk

- HIGH - Solid wood
  - Raw logs, round wood poles, timbers, lumber
- MEDIUM - Wood fragments
  - Sawdust, chips, shavings, fuel pellets, flour
- LOW - Wood composites
  - Plywood, OSB, fiberboard, engineered wood



ISPM-15

- Heat treatment 56C for 30 min to the core
- MeBr fumigation under specific guidelines

Current Work Relevant to ISPM-15

- Calls for submissions of new treatments (2006, 2007)
- Efficacy of accepted treatments (HT, MeBr)
- Alternative treatments - protocols
  - Fumigation with MeBr replacements
  - Microwave/Radio Frequency
  - Irradiation
  - Chemical Pressure Impregnation
  - Others?

Questions on How to Get Satisfactory Data?

- How to test efficacy?
- Naturally infested wood vs lab inoculated, size, 1 specimen in 1 test piece of many in 1 test piece, substrate mc, frozen wood, with/without bark, which organisams (strain, developmental stage) and replication, how to assess data, what is acceptable statistics?

A Question!

- If you use 10 insects in your test and manage to kill all of them using a particular treatment parameters, does that make you a 100 % efficaceous?

Efficacy / Reliability Defined

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. Probability that infected piece is successfully treated. Expressed as decimal portion of 1.0.

Common Statistical Formula

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

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

n	ln(.05)/n	prely=exp(ln(.05)
5	-0.599146455	**0.549280272**
10	-0.299573227	**0.741134449**
15	-0.199715485	**0.818963727**
20	-0.149786614	**0.860891659**
25	-0.119829291	**0.887071855**
30	-0.099857742	**0.904966147**
35	-0.085592351	**0.917968364**
40	-0.074893307	**0.927842475**
45	-0.066571828	**0.935595711**
50	-0.059914645	**0.941844921**
59	-0.050775123	**0.95049239**
60	-0.049928871	**0.951297087**
100	-0.029957323	**0.97048695**

This is also called a BRUTE-FORCE METHOD

What is Acceptable Reliability ?

- Probability of 1.0 is impossible to achieve
- Probit 9 treatments require 0.99997 reliability
  - It was/is a standard for required quarantine treatments reliability
  - Originate from the statistical method (Probit analysis) used to derive dose response relationship. It provides adequate quarantine security. Set originally for tropical insects (high levels of infestation) in tropical fruits (high volume of commodity)
  - You need 93,613 insects to test without survivors to meet probit 9
  - Probit 9 is far too stringent for commodities that are rarely infested or are poor host (PRA)

Two Methods to Test Reliability

If you want to determine the reliability of a selected dose two types of experiments can be conducted:

- BRUTE FORCE EXPERIMENTS - Reliability of none found alive
- Involves exposing n number of units to the selected dose and no units should have any survivors
  - 0.95% effective (95% confidence) n=59
  - 0.99% effective (95% confidence) n=299
  - 0.999% effective (95% confidence) 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

Case Study – PWN 56° / 30min

- Forintek (FPInnovations) was commissioned by the Canadian Federal Goverment to lead a cooperative research program to examine HT as alternative to KD for the eradication of PWN and its vector

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, frankly, not expected

- 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

Approach

A series of preliminary experiments were undertaken to identify the most heat resistant Canadian softwood species and PWN isolate under the worst-case wood moisture content for heat treatment of PWN in wood (worst case scenario).

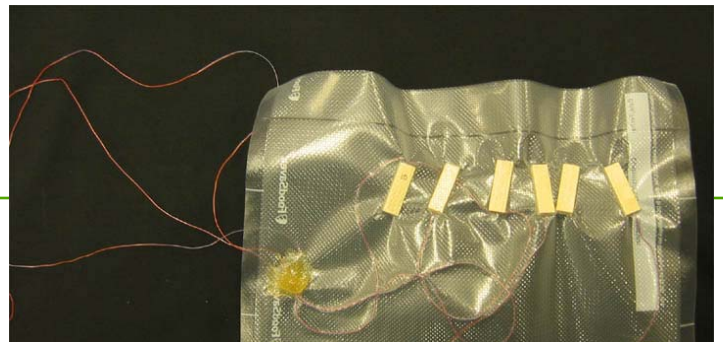
The results were used to design an experiment

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



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Results

Temperature	Number of Blocks	Number of blocks with 100% dead PWN
25	60	1
35	60	6
40	60	16
45	60	34
50	60	59
52	59	59
54	60	60
56	60	60
60	60	60
62	60	60
64	60	60
66	60	60

Fit your data to a dose response models and do goodness fit tests to help you chose one (Finney, D.J. 1971. Probit Analysis).

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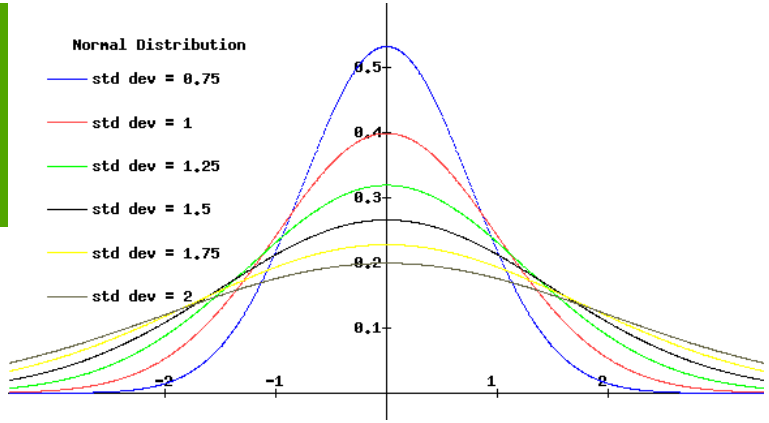
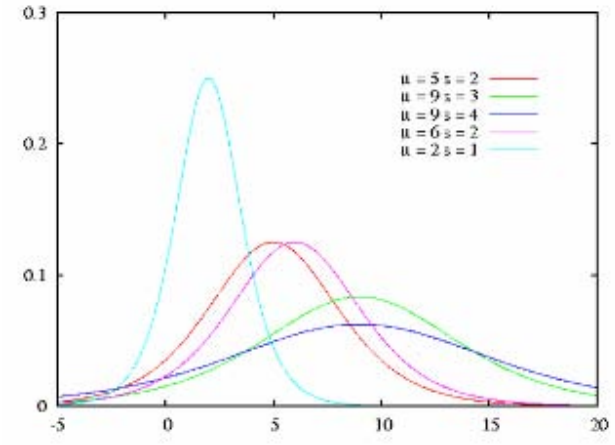
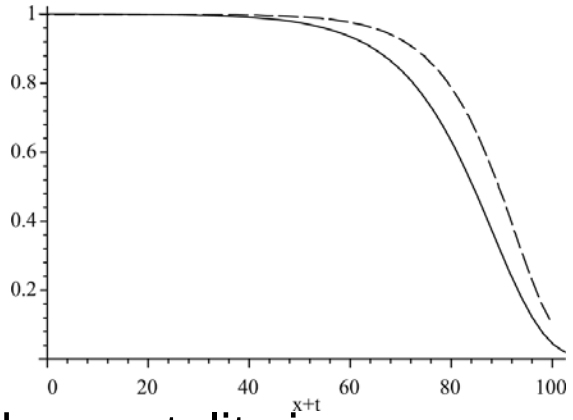
Three Distributions

- 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)

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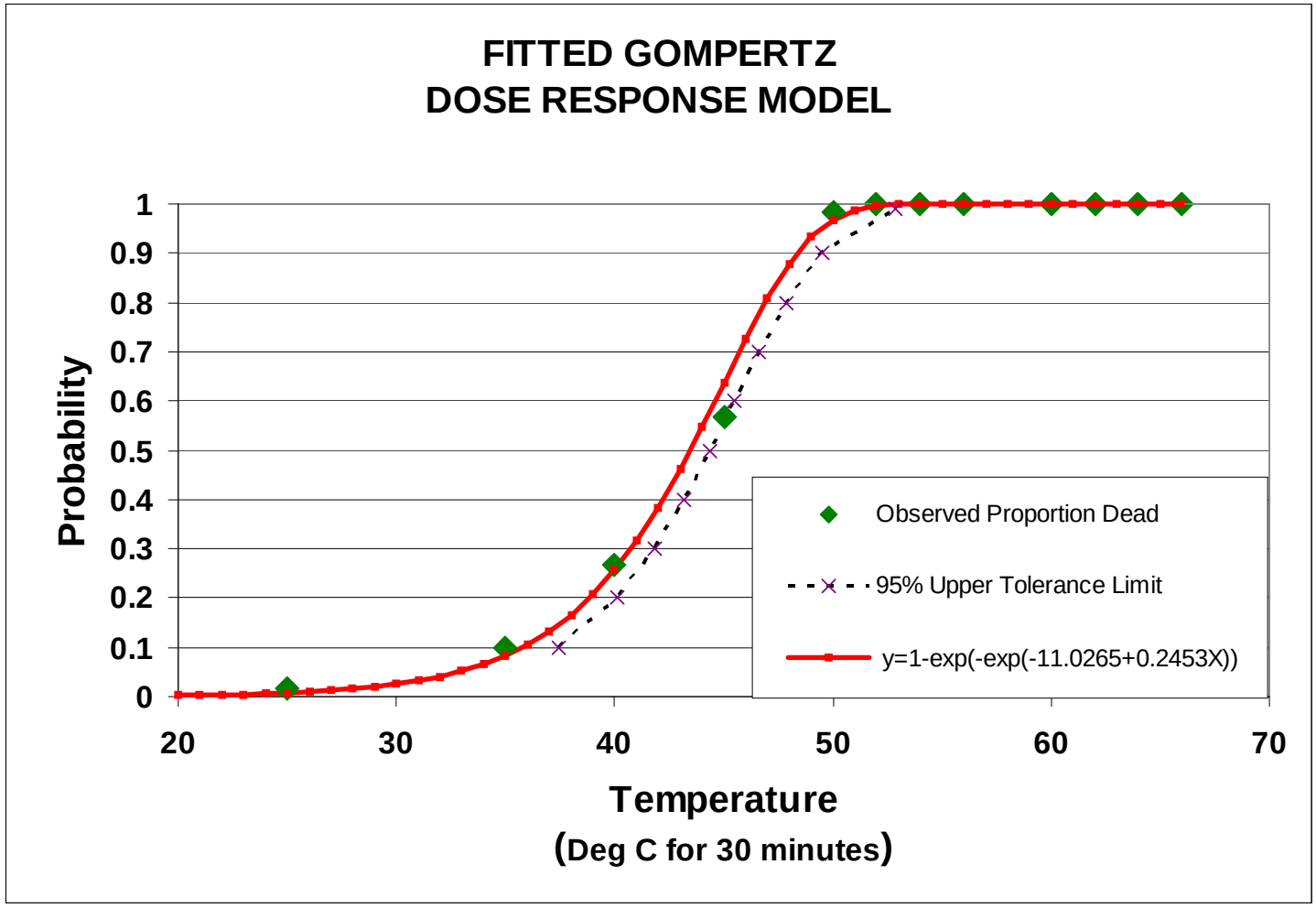
Results

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



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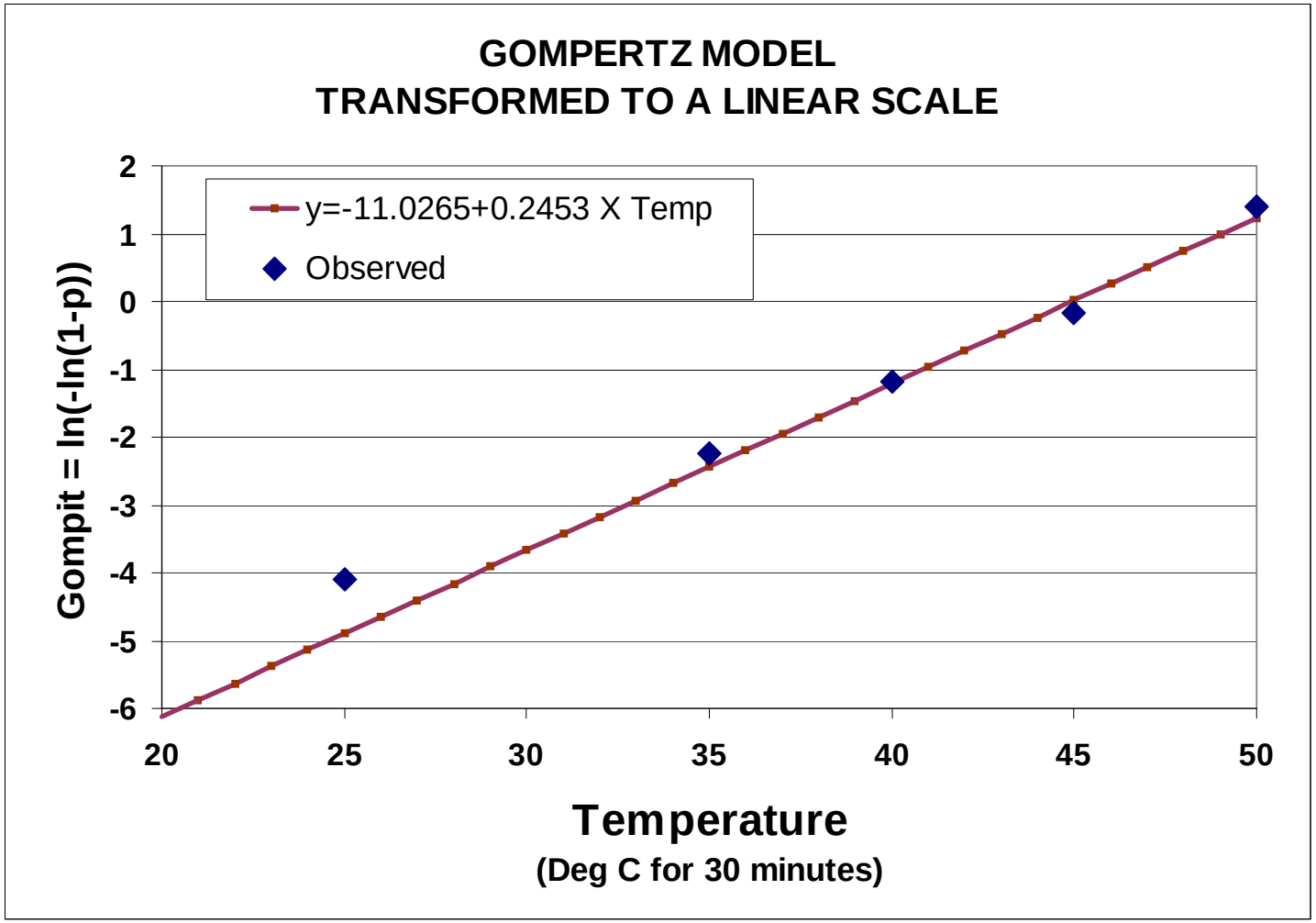
Gompit



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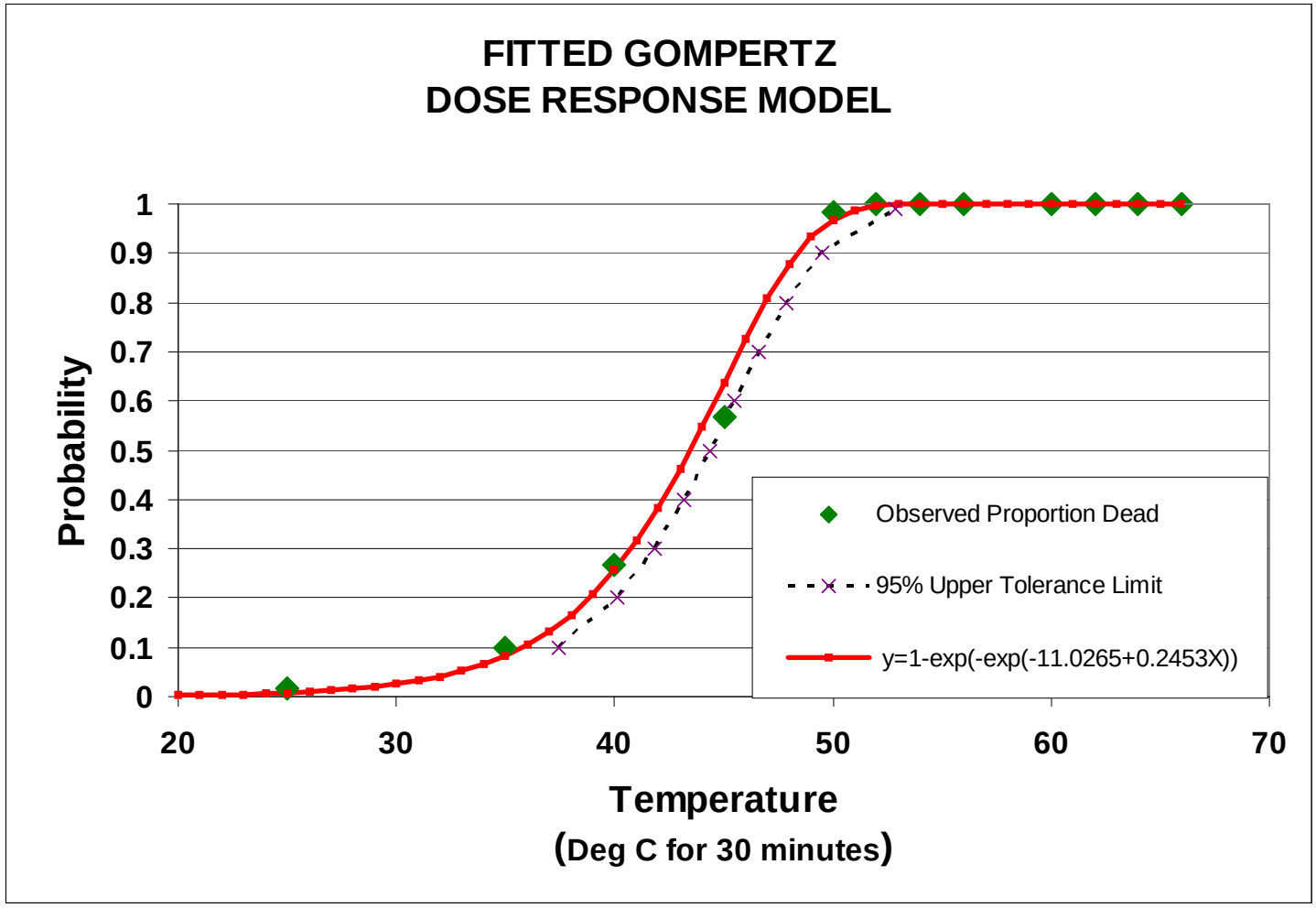
Mortality Curve



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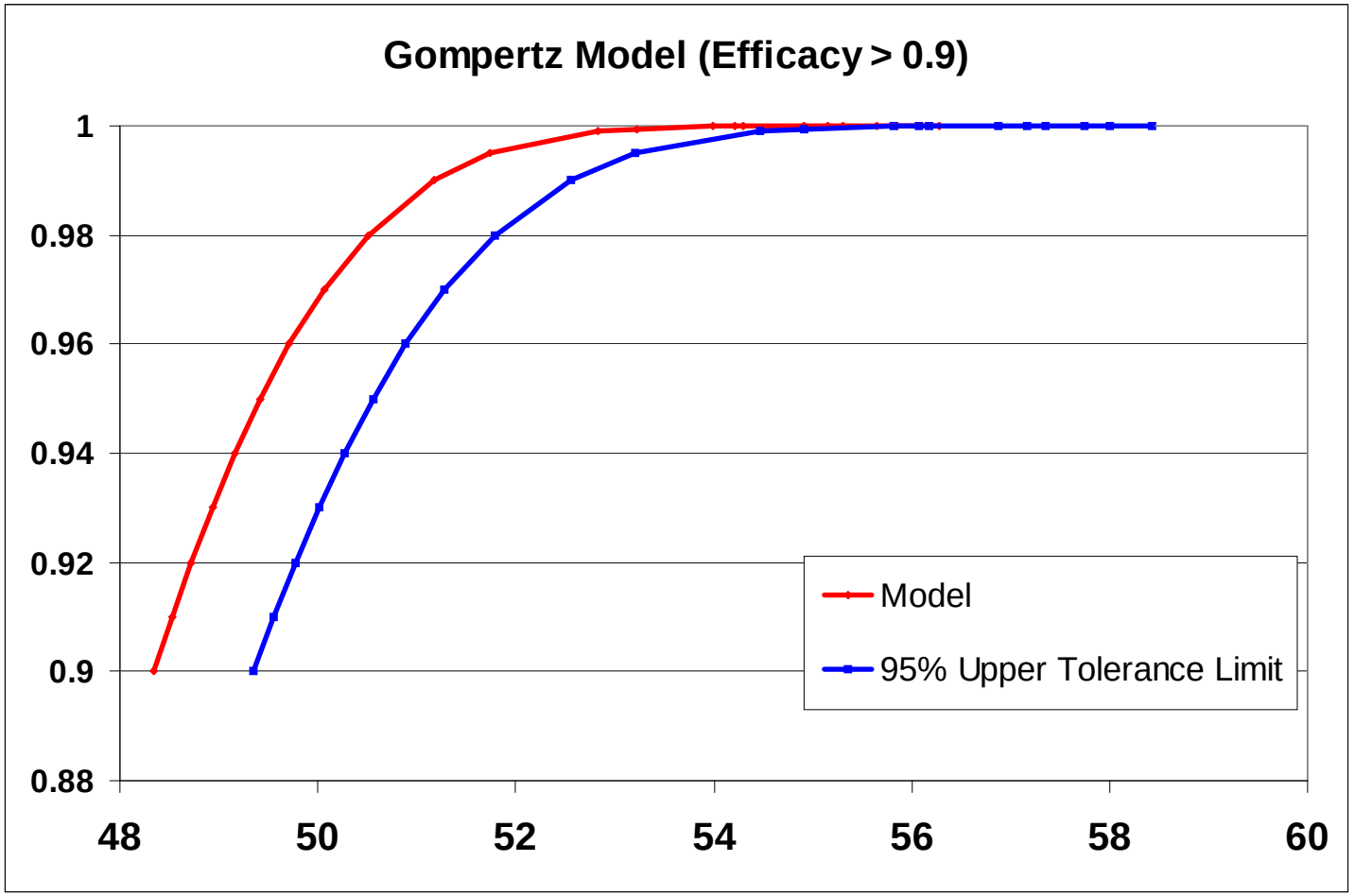
Blown up part of the Curve Where Efficacy > 0.9



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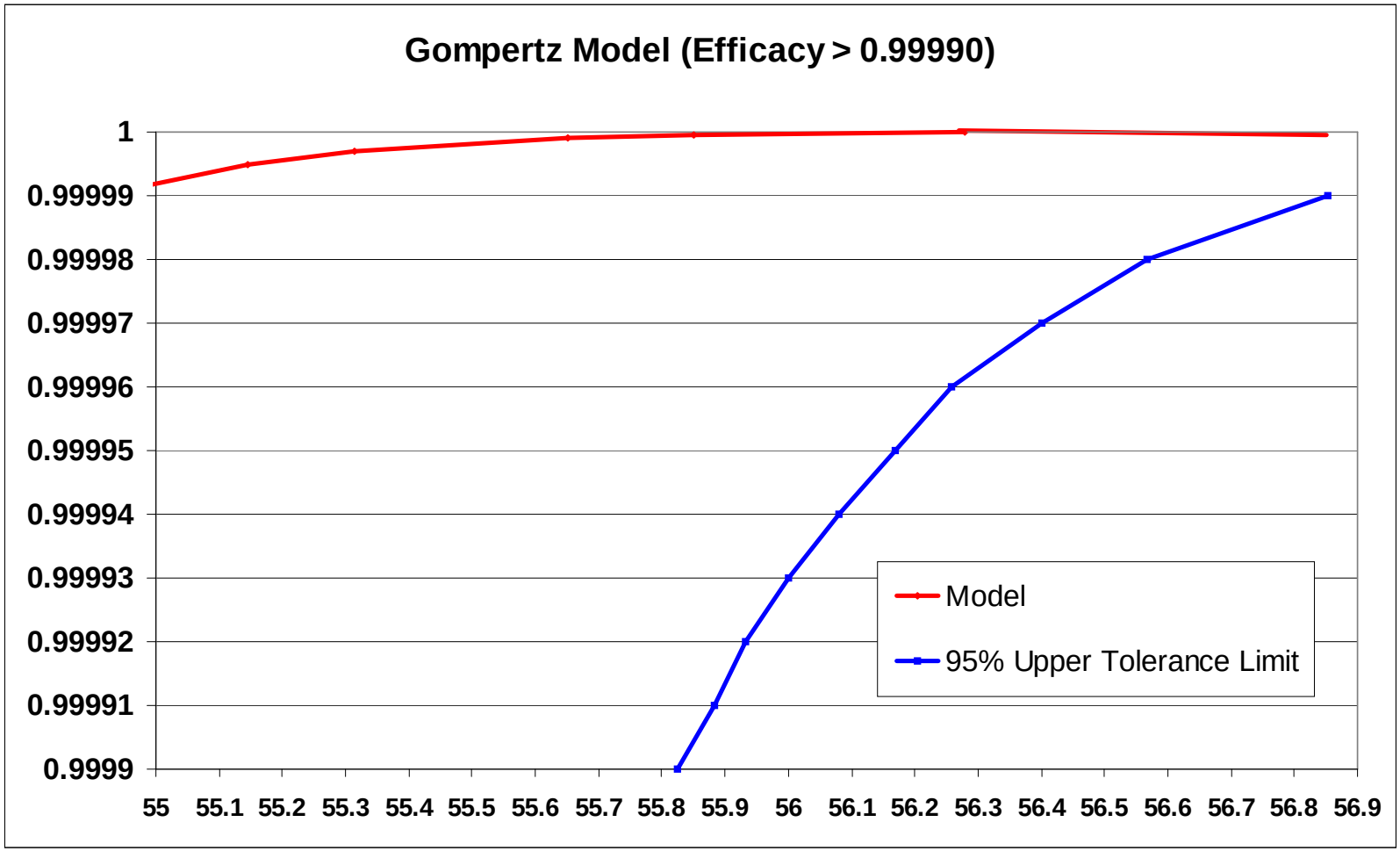
Various Thermal Death Times for PWN in Canadian Softwood Lumber; Efficacy > 0.9999



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What is a Reasonable Target for Efficacy?

The short answer:

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

Development of Alternative Treatments-Protocols

- IFQRG Rome September 2007, we were divided into 3 groups (insects, fungi, nematodes) and asked:
  - To compose shortest list of pests that should be tested in order to approve treatment and convince NPPOs and CPM
  - Reasoning for the choice
  - Prevalence in choice test pests in wood
  - Number of replication needed
  - What aspects of the organism occurrence in wood should be considered for appropriate treatment

Development of Alternative Treatments

Insect group:

30 units per treat. parameters

Cerambycids/Buprestids (L)

Siricids, Cossids

Scolitidae, Curculionidae, Platypotidae (L, A)

Isoptera (A)

Lychtyds/Botrichids, Anobiidae (L)

Fungi group:

60 units (3 isolates)

Decay fungi (5 species)

Canker causing fungi (4)

Wilt fungi (2)

Bluestain fungi (4)

Nematodes group:

- 100,000 nematodes
- Follow PWN work 56/30

Bursaphelenchus xylophilus

B. mucronatus

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Recommendations of TPPT (Dec 2007)

A two stage process

- 1st-Fourteen different pests including **key species** need to be tested to determine most resistant pest and life stage
- 2nd large scale testing of the most resistant pest (life stage) under operational conditions

…the extrapolation may be the only practical way of evaluating efficacies for pests that did not occur in large quantities (e.g. wood borers)

…The panel discussed if Probit 9 level is always necessary…it is appropriate to set a high standard , effectiveness.

Issues

- Raising bar too high might preclude new treatments to emerge
- Research guidelines are still vague
- Reliability levels need to be agreed and understood
- Can we settle with fewer species and/or use reference pests.
- Pure laboratory data versus real life situation (naturally infested)
- Odd, unexpected data

Heat Treatment of Inoculated Pure Fungi-Results

- 15 species tested (include bluestain, decay and ambrosia fungi). Three isolates/species replicated six times

- 1 bluestain (4 isol.) and
- 4 decay fungi (18+18+16+18 isol.) surivived 56/30 but were all killed at the next temperature 61/30

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Fungi in Naturally Infested Wood – HT (% survival)

Temp	Time	Stain		Yeast	Mold					Decay
		Sporothx	Leptogr		Zygos	Asperg	Paecilo.	Penicil.	Trichod.	
41	**1** **30** **60** **120**		100 100 70 70	80 100 100 100	10	10 10	10	50 40 30	20 30 40 30	80 20 30 60
46	**1** **30** **60** **120**		100 20 20	100 60 40 20		10		20 10 10	10 30 10	30 20 0
51	**1** **30** **60** **120**		20	80 10 10			10		40	20
56	**1** **30** **60** **120**			20		10				
61	**1** **30** **60** **120**					10		10		
66	**1** **30** **60** **120**			10				10		
71	**1** **30** **60** **120**			20						

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Results-Conclusions-Naturally Infested Wood

- *Trichoderma, Penicillium, Aspergillus etc* overtook completely heated naturally-infested test wood

Questions, Comments, Thoughts?

What data would satisfy you to accept that a treatment can provide global level of protection?

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