

## Seeking alternatives to probit 9 when developing treatments for wood packaging materials under ISPM No. 15

R. A. Haack<sup>1</sup>, A. Uzunovic<sup>2</sup>, K. Hoover<sup>3</sup> and J. A. Cook<sup>2</sup>

<sup>1</sup>USDA Forest Service, Northern Research Station, 1407 S. Harrison Road, East Lansing, MI 48823 (USA); e-mail: rhaack@fs.fed.us

<sup>2</sup>FPIInnovations, 2665 East Mall, Vancouver, BC V6T 1W5 (Canada); e-mails: Adnan.Uzunovic@fpinnovations.ca and Jean.Cook@fpinnovations.ca

<sup>3</sup>Department of Entomology, The Pennsylvania State University, 501 ASI Building, University Park, PA 16802 (USA); e-mail: kxh25@psu.edu

ISPM No. 15 presents guidelines for treating wood packaging material used in international trade. There are currently two approved phytosanitary treatments: heat treatment and methyl bromide fumigation. New treatments are under development, and are needed given that methyl bromide is being phased out. Probit 9 efficacy (100% mortality of at least 93 613 test organisms) has been suggested as an evaluation criterion for new wood treatments, and is based on fruit fly research. We question requiring probit 9 efficacy for wood pests (insects, nematodes and fungi) and discuss challenges to meeting this requirement. Instead, we suggest a 3-step, laboratory-based alternative approach. Step 1 involves laboratory experiments (screening) to estimate the lethal dose for the most tolerant stage of each target pest. We consider each infested piece of wood as an experimental unit, not the individual pests, to avoid pseudoreplication. Step 2 requires replicated experiments (with no survivors) at the estimated lethal dose. We suggest a minimum sample size of 60 experimental units, which achieves 0.95 statistical reliability at the 95% confidence level. Step 3 entails studies under simulated operational conditions using wood samples similar in size to wood packaging material and infested to levels that reflect field conditions.

### Introduction

Many species of insects, nematodes and fungi colonize living and recently dead trees throughout the world. When infested trees or logs are converted into wood packaging material such as crates, dunnage and pallets used in international trade, there is potential for pests to be moved inadvertently to new countries (Brockhoff *et al.*, 2006; Haack, 2006; McCullough *et al.*, 2006; Zahid *et al.*, 2008; Roques *et al.*, 2009; Haack *et al.*, 2010). In recognition of this high-risk pathway, in 2002 the world community adopted International Standards for Phytosanitary Measures (ISPM) No. 15, entitled 'Guidelines for Regulating Wood Packaging Material in International Trade' (IPPC, 2002). When ISPM No. 15 was originally written (IPPC, 2002), and when last revised (IPPC, 2009a), the only two approved phytosanitary treatments were heat treatment to a minimum temperature of 56°C for 30 min (56/30) throughout the entire profile of the wood, and methyl bromide fumigation following schedules prescribed in the standard (IPPC, 2009a).

The original 56/30 schedule was based on extensive laboratory studies in Canada that determined the time-temperature combination that was lethal to the pinewood nematode (*Bursaphelenchus xylophilus*; Smith, 1991, 1992). This work was funded by a government-industry consortium in Canada to facilitate trade of North American lumber to Europe. A series of preliminary experiments were conducted to examine heat tolerance in

pinewood nematode relative to nematode strain, tree species and wood moisture content. Subsequent testing, using samples that represented the worst case conditions, was conducted with the goal of achieving 100% mortality at a reliability of 0.99994. The data were analysed using extrapolation and probit-like analysis (Finney, 1971). Years later, the 56/30 schedule was adopted as the standard for heat treatment under ISPM No. 15 for basically all wood pests associated with wood packaging material (IPPC, 2002, 2009a).

Currently, there is great interest in developing new technologies to treat wood packaging material for inclusion in ISPM No. 15, especially new fumigants, given that use of methyl bromide is being phased out worldwide. When developing new treatments it is important to know the level of mortality or effectiveness (see terminology below) that the new treatment must achieve to be considered for approval.

The Commission on Phytosanitary Measures serves as the governing body within the International Plant Protection Convention (IPPC), and is responsible for development and adoption of ISPMs. In addition, there are several committees, technical panels and expert working groups within the IPPC that assist in developing and revising ISPMs. The Technical Panel on Phytosanitary Treatments (TPPT) has the lead responsibility for evaluating submissions for ISPM treatments. With regard to ISPM No. 15, the TPPT requested in 2007 that the Technical Panel on Forest Quarantine (TPFQ) develop evaluation criteria, including a list of

target pests and the required level of efficacy that included consideration of probit 9 (TPFQ, 2008). The purpose of this paper is to discuss the history of probit 9 and the challenges of achieving it with respect to wood-infesting organisms, and to suggest an alternative approach to probit 9 when developing treatments for ISPM No. 15.

### Goals of quarantine treatments and ISPM No. 15

The goal of quarantine treatments is to eliminate or minimize the risk of pests being spread through traded commodities, including wood packaging material (Landolt *et al.*, 1984; Roth, 1989). Quarantine treatments are generally classified as chemical (e.g. fumigants) or physical (e.g. heat, cold and irradiation), and are used to sterilize or kill regulated pests that are on or in the commodity at the time of treatment (Follett & Neven, 2006). The goal of ISPM No. 15 is very similar. As first written in 2002, and again in the 2006 revision, the stated goal of ISPM No. 15 was to 'practically eliminate the risk for most quarantine pests and significantly reduce the risk from a number of other pests that may be associated' with wood packaging material (IPPC, 2002). In the 2009 revision of ISPM No. 15, the stated goal was changed slightly to 'reduce significantly the risk of introduction and spread of most quarantine pests' (IPPC, 2009a). It is important to keep this revised wording in mind and recognize that the goal of ISPM No. 15 is not zero risk, but rather significantly reduced risk.

### Efficacy testing for ISPM No. 15 treatments

Guidelines for developing new or revised phytosanitary treatments are given in ISPM No. 28: 'Phytosanitary Treatments for Regulated Pests' (IPPC, 2009b). The guidelines presented in ISPM No. 28 are very general: the document simply states that efficacy data must be submitted by the treatment developer but does not specify the level of efficacy required for approval. However, in early 2010, a new draft appendix to ISPM No. 15 was released for country consultation that listed specific guidelines for researchers to follow when developing new treatments for possible inclusion in ISPM No. 15 (IPPC, 2010). One guideline in this draft document stated that probit 9 efficacy should be demonstrated for the target pests either by direct testing (i.e. treating at least 93 613 individuals with 100% mortality) or through extrapolation based on dose–response data.

### History, benefits and criticisms of probit 9

The concept of probit was first published by Bliss (1934) as a means to express percent mortality data by dividing the range 0.01 to 99.99% into probability units or probits where 0 = 0.01% kill, 5 = 50% kill, and 10 = 99.99% kill. Probit analysis assumes that the variation in tolerance of the target organisms to a given dose is normally distributed (Bliss, 1934; Liquido *et al.*, 1997; Robertson *et al.*, 2007).

Today probit 9 equates to mortality of 99.9968% (Baker, 1939; Follett & Neven, 2006; Robertson *et al.*, 2007). Although

Baker (1939) selected probit 9 as the required level of efficacy for quarantine treatments against fruit flies (Tephritidae), probit 9 has now been widely adopted by the United States and many other countries as the benchmark for approving quarantine treatments for a wide variety of pests (Roth, 1989; Liquido *et al.*, 1997; Follett & Neven, 2006). Probit 9 is listed as the basis for several quarantine treatments in the USDA Animal and Plant Health Inspection Service (APHIS) treatment manual (USDA APHIS, 2010). Baker (1939) provided no rationale for selecting mortality as the evaluation criterion or for selecting probit 9, except to state that his objective was to 'assure no survival of (fruit fly) eggs or larvae in the products treated'. If one sets the probability of obtaining this efficacy level by chance alone at 5% (95% confidence level), at least 93 613 insects must be tested without any survivors to attain efficacy of probit 9 (Couey & Chew, 1986; Follett & Neven, 2006). Obtaining large numbers of fruit flies is achievable, given their short generation time and availability of efficient rearing methods.

Probit 9 has some advantages, but it has also been criticized. The principal advantages of probit 9 include the apparent high degree of quarantine security and the relative ease of convincing a trading partner to accept a treatment that achieves probit 9 efficacy (Follett & Neven, 2006). The main criticisms directed at treatments for which probit 9 efficacy is required are that (1) substantial numbers of live pests can still be shipped on treated commodities when trade volume or infestation levels are high, given that probit 9 can be viewed as either 99.9968% mortality or 0.0032% survival; (2) for products rarely infested, requiring probit 9 is often considered too severe and possibly difficult to demonstrate; (3) other models besides probit are available to analyse dose–response data (e.g. logit, log-log or Gompertz) and these often give a better fit to the data than the normal distribution; and (4) the focus on mortality as the sole criterion for evaluating quarantine security disregards risk-based factors along the pathway, such as the likelihood of infestation, natural survival, reproductive potential and establishment potential, as well as processing parameters such as packaging and shipping practices and distribution times (Landolt *et al.*, 1984; Liquido *et al.*, 1997; Follett & Neven, 2006; Robertson *et al.*, 2007). Other approaches to evaluating pest risk have been proposed in recent decades as alternatives to probit 9, especially for horticultural products, which focus on reducing pest incidence below the threshold for establishment, such as the use of pest-free areas, system approaches, and maximum pest limits (Landolt *et al.*, 1984; Baker *et al.*, 1990; Liquido *et al.*, 1997; Follett & Neven, 2006). Even though probit 9 is recognized as having weaknesses and alternatives have been presented to the phytosanitary community, no other method has gained wide acceptance and regulatory recognition to date (Follett & Neven, 2006).

### Key terminology used in discussing treatments

The terminology used in the development and assessment of treatments can be vague and may be misinterpreted. The word 'efficacy', in particular, means different things to different people

and is often confused with ‘effectiveness’, ‘confidence’ or ‘reliability’. Precise understanding of these terms is critical for clear stipulation of treatment criteria. Efficacy is used throughout ISPM No. 28, (IPPC, 2009b), and the draft appendices to ISPM No. 15 (IPPC, 2010). Efficacy is often defined as the ability (or capacity) to produce a desired effect. It is not a statistical term. Efficacy of probit 9 is not equivalent to 99.9968% confidence that the lethal dose is indeed what was determined experimentally; instead, it is the dose that produces mortality of 99.9968% in a population of, say, 100 000 individuals. More importantly, efficacy refers to results obtained under ideal treatment conditions, similar to rigorous clinical trials, whereas effectiveness refers to results obtained under real-world treatment conditions. Researchers generally first examine the efficacy of a treatment in controlled trials (see Steps 1 and 2 below) and then conduct studies to determine if the treatment is equally effective under operational conditions (see Step 3 below).

In contrast, confidence and reliability have statistical meanings. Typically, biologists set Type I error ( $\alpha$ ) at 0.05, which provides a 95% confidence interval around the mean ( $1.0 - 0.05 = 0.95$  or 95%). This means that you can be 95% confident that the true mean lies within this interval (the mean  $\pm$  a degree of error), while 5% of the time it does not. We use the term ‘statistical reliability’ as the probability that the same result will be obtained again and again with repetition. Thus reliability is a term that refers to the level of trust in the results, and is dependent on sample size and variability in the data. We suggest using reliability (or statistical reliability) rather than efficacy when referring to the ability to produce similar results over time (see Step 2). In addition, we consider each infested piece of wood as an experimental unit regardless of the number of pest organisms within (see Step 2).

### Diversity of wood-inhabiting organisms

When ISPM 15 was first approved in 2002, it was focused on several families of bark- and wood-infesting insects such as Buprestidae, Cerambycidae, Scolytidae and Siricidae, as well as the pinewood nematode (IPPC, 2002). In the 2010 draft appendix to ISPM No. 15 (IPPC, 2010), fungi were added to the list of organisms against which new treatments should be evaluated.

Worldwide, there are thousands of insect species that colonize and develop in the bark and wood of trees. The numbers of species found in several important insect families whose members commonly infest bark and wood are presented in Table 1. Some of these insects colonize live, apparently healthy trees, others colonize only stressed or recently dead trees, and others infest only dry or decomposing wood (Haack & Slansky, 1987; Hanks, 1999; Lieutier *et al.*, 2004). We did not find summary data for the number of wood-inhabiting fungi and nematodes known worldwide, but undoubtedly there are several thousand species (Callan & Caris, 2004; Ryss *et al.*, 2005; Unterseher *et al.*, 2008). Moreover, there are likely to be hundreds of species of wood-inhabiting organisms that are still undescribed worldwide. Nevertheless, only a small percentage of these organisms actually cause tree death, such as *Anoplophora glabripennis* (Asian longhorned beetle), *Agrilus planipennis* (emerald ash borer) and

**Table 1** Number of insect species found in the principal families of Coleoptera, Hymenoptera and Lepidoptera that commonly inhabit bark and wood of woody plants

| Order       | Family        | Approximate number of species known in: |             |          |
|-------------|---------------|---|-------------|----------|
|             |               | World*                                  | N. America* | Europe*  |
| Coleoptera  | Anobiidae     | 2 200 (b)                               | 402 (i)     | 434† (d) |
|             | Bostrichidae  | 550 (b)                                 | 77 (b)      | 44 (d)   |
|             | Buprestidae   | 14 600 (b)                              | 762 (b)     | 437 (d)  |
|             | Cerambycidae  | >20 000 (b)                             | >900 (b)    | 695 (c)  |
|             | Platypodidae‡ | 1 463 (m)                               | 7 (b)       | 3 (k)    |
|             | Scolytidae‡   | 5 812 (m)                               | 581 (b)     | 376 (k)  |
| Hymenoptera | Siricidae     | 100 (l)                                 | 20 (l)      | 21 (j)   |
| Lepidoptera | Cossidae      | 682 (g)                                 | 45 (a)      | 23 (f)   |
|             | Sesiidae      | 1 325 (g)                               | 123 (e)     | 115 (h)  |

\*References: a = Arnett (2000), b = Arnett *et al.* (2002), c = Cocquemot & Lindelöw (2010), d = Denux & Zagatti (2010), e = Eichlin & Duckworth (1988), f = Fauna Europaea (2010), g = Heppner (2008), h = Lopez-Vaamonde *et al.* (2010), i = Poole & Gentili (1996), j = Rasplus *et al.* (2010), k = Sauvard *et al.* (2010), l = Schiff *et al.* (2006), m = Wood & Bright (1992).

†The number of European Anobiidae (434) includes 421 species that were classified as Anobiidae plus 13 species of Lyctidae. The Lyctidae are considered by many as a subfamily of the Anobiidae. The anobiid values given for the world and North America include the Lyctinae.

‡The beetle families Platypodidae and Scolytidae are currently recognized by many as subfamilies of the weevil family Curculionidae, but most plant health regulatory organizations worldwide still treat them as distinct families.

*Bursaphelenchus xylophilus* (pinewood nematode) (Ryss *et al.*, 2005; Haack, 2006; Haack *et al.*, 2010). Given such diversity, it is clear that only a small fraction of all wood pests can be screened and tested when developing new treatments for ISPM No. 15.

### Life-history attributes of wood-infesting pests relevant to probit 9

Among the numerous factors that influence the overall risk of pest movement in trade, life history characteristics can provide guidance for selecting the appropriate level of treatment efficacy. The efficacy level needed for a given pest can be estimated through assessment of biological characteristics that affect the likelihood that an organism will be introduced and become established, including fecundity, longevity, voltinism, parthenogenesis (if relevant), prevalence in wood, dispersal ability, vector relationships (if relevant), host range, founder population dynamics, sporulation characteristics of fungi (asexual and sexual reproduction), resting stages and sublethal effects. Assessment of these characteristics should provide insight into the acceptable number of organisms that can survive a treatment and still provide acceptable phytosanitary security.

As mentioned above, probit 9 was originally proposed as a statistical approach to evaluate treatments for fruit flies. However, in contrast to fruit flies, which are relatively easy to mass-rear, bark- and wood-infesting insects present many challenges, making it impractical to achieve sample sizes of nearly 100 000. For

example, naturally infested host material must be used for many borers, which requires considerable effort to locate and cut infested trees, and then transport the infested logs to the laboratory for processing and testing. In addition, natural borer infestation rates can vary widely from year to year, from tree to tree, and even within a single tree. Based on our rearing experience with well infested logs, it would be common for a log that measures about 1 m long and 10 cm in diameter to contain about 100–250 individual bark beetles such as the pine shoot beetle (*Tomicus piniperda*), 20–30 buprestids such as the emerald ash borer, or 5–10 cerambycids such as the Asian longhorned beetle. Therefore to obtain 93 613 individuals for probit 9 testing with logs of similar size (1 m × 10 cm) and infestation levels, a researcher would need 374–936 bark beetle-infested logs, 3120–4681 buprestid-infested logs, or 9361–18 723 cerambycid-infested logs. Another challenge to working with many borers is their long generation time. In contrast to fruit flies, which can often complete one generation per month, most bark beetles require 2–3 months per generation, while others require a year or more. Similarly, many buprestids and cerambycids (such as the emerald ash borer and Asian longhorned beetle) can complete one generation per year, while other species usually require 2–3 years or more per generation (Haack & Slansky, 1987; Haack, 2006). Because most testing is conducted when the target insects are larvae, it is necessary to store the test logs in specialized rearing containers for several months to ensure adequate time for adult emergence. Clearly, few facilities could treat and store the number of test logs necessary to achieve probit 9 testing, and even fewer if the target organism must be tested within a quarantine facility.

*Bursaphelenchus xylophilus* (pinewood nematode) is the nematode of principal concern to forestry worldwide (Ryss *et al.*, 2005). This organism is relatively easily to culture in the laboratory in large numbers and therefore obtaining 100 000 organisms is achievable.

On the other hand, wood-colonizing fungi are more difficult to work with because they are not easily defined, discrete organisms like a single insect or nematode. Because fungi grow within the wood matrix, each individual wood block would need to be counted as an individual, thus to meet probit 9, a researcher would need to test at least 93 613 individual pieces of wood that have been colonized by the target fungus. Another option would be to treat single fungal spores as discrete individuals, which would allow a researcher easily to meet probit 9, but we question the validity of evaluating the survival of spores rather than other fungal structures as a measure of treatment success or failure.

## Extrapolation

The draft appendix for ISPM No. 15 (IPPC, 2010) recognized that it would often be difficult to obtain 93 613 organisms for testing and therefore allowed use of extrapolation to estimate the dose required to achieve probit 9 efficacy. Although this allowance greatly reduces the burden on treatment developers, it could have negative consequences. For example, when modeling or extrapolating from dose–mortality response data, the dose estimated through extrapolation overestimates what would have been

the experimentally derived dose (Smith, 1991; Hoover *et al.*, 2010). Overestimating the lethal dose will result in overtreatment, which could result in increased manufacturing costs, increased environmental impacts (e.g. larger carbon footprint), and possible damage to the wood itself with some treatments (e.g. heat).

## An alternative approach to probit 9 for ISPM No. 15

We present a three-step process for consideration as an alternative to probit 9 when developing treatments for ISPM No. 15. Briefly, Step 1 involves small-scale laboratory experiments that allow estimation of the lethal dose to the target pest, focusing on the most tolerant life stage. Step 2 involves replicated experiments at the estimated lethal dose to provide statistical confidence and establish reliability. For Step 3, a scaled-up confirmatory study would be conducted that involves testing wood of a size that is representative of wood packaging material and demonstrates that the treatment can be effectively applied operationally.

### Step 1. Estimating the lethal dose of the most tolerant life stage

One of the first steps in treatment development is to estimate the treatment dose for the target pest at which all or nearly all organisms die (i.e. the lethal dose). The draft appendix to ISPM No. 15 states that the sample size for determining the lethal dose could be 5–10 experimental units per dose (IPPC, 2010). However, this sample size may not be sufficient, depending on the degree of variability observed. Robertson *et al.* (2007) published an entire book on this topic and have developed software that analyses dose–response data using probit or logit regression (LeOr Software 2007). When selecting target species for Step 1, researchers need to consult ISPM No. 15 and its appendices to learn which pest species or pest groups are currently of high quarantine importance, and if guidelines are provided as to how many species should be tested from each major pest group (insects, nematodes and fungi). The researcher also needs to know which life stages of the target pest are present in traded commodities, and then to focus on the life stage most tolerant to the proposed treatment (IPPC, 2009b). Such information may be available in the literature, or it may require preliminary testing. We also suggest testing the smallest wood samples that are practical for the target pest being studied to ensure the dose is delivered uniformly throughout the experimental unit. In large-dimension experimental units, such as infested logs, it may be difficult to precisely deliver the dose uniformly, therefore confounding the results if there are survivors.

### Step 2. Replicated experiments at the estimated lethal dose

In Step 2, we propose that the treatment developer should test sufficient experimental units at the estimated lethal dose (without survivors) for each target pest to obtain a reliability of 0.95 at the 95% confidence level, and if possible should also test one or two doses above and below the estimated lethal dose. The actual

sample size required is a matter for the international community to discuss. It is well documented that, as sample size increases, the size of confidence interval decreases and reliability increases. Table 2 shows the relationship between sample size and statistical reliability when expressed in terms of 95% confidence (Beyer, 1968). For example, if only 5 samples are tested and there are no survivors, the reliability of these data, expressed with 95% confidence, is 0.549, meaning that there is a 54.9% probability that the dose will be lethal. However, with 60 samples, the reliability increases to 0.951; similarly, with 100 and 299 samples, the reliability increases to 0.970 and 0.990, respectively (Table 2). Considering the many challenges of working with wood-infesting organisms, we suggest that sample sizes in the range of 60–100 be considered, given that they equate roughly to 0.95 and 0.97 reliability. Clearly, a reliability of 1.0 is impossible as this would require a sample size that approaches infinity.

A sample size of 93 613 equates to a reliability of 0.999968 (Table 2), which would require testing 93 613 samples with no survivors. This is the same value as the specified treatment efficacy in probit 9 (Couey & Chew, 1986; Follett & McQuate, 2001). However, we argue that treatment efficacy should not be confused with statistical reliability, and researchers and regulators should strive for a reasonable level of reliability of the data (i.e. obtaining highly similar results with repeated testing). A sample size of 93 613 is obviously an overwhelming task, and if required could impede development of new treatments.

The 2010 draft appendix to ISPM No. 15 (IPPC, 2010) allows treating 93 613 individuals in a single piece of wood as satisfying the probit 9 requirement when there are no survivors. However, in our opinion, if all 93 613 individuals are in a single piece of wood, then the true sample size is 1 ( $n = 1$ ) which equates to a reliability of 0.05 (Table 2). We consider counting each individual pest in a single wood sample as a form of pseudoreplication. In statistics, pseudoreplication refers to taking multiple measurements on the same sample unit and treating each measurement as independent, when in fact they are probably interdependent. We

suggest that the responses of all organisms within a single block of wood would be interdependent because nearly all would receive a similar dose. Therefore a single test block of wood should be considered as the experimental unit, no matter how many test organisms it contains. In practice, researchers never really know how many organisms are inside field-collected wood.

### Step 3. Confirmatory study under simulated operational conditions

In Step 3, we propose that a confirmatory study be conducted under simulated operational conditions in which the treated wood samples are similar in size to typical wood packaging material. Recalling that reliability increases with sample size (Table 2), we suggest that the confirmatory studies be conducted with a sample size linked to the perceived phytosanitary risk of the target pest. For example, 300 samples (0.99 reliability; Table 2) could be requested for high-risk pests such as pinewood nematode and Asian longhorned beetle, which can infest and kill healthy trees, but 60 samples (0.95 reliability) may be adequate for quarantine pests that usually infest stressed trees, like many bark beetles. The wood samples should be prepared from naturally infested trees, or, if necessary, pest organisms could be introduced into each wood sample to ensure sufficient numbers are present. If there are survivors in these confirmatory studies, then it is possible that the treatment was not delivered uniformly throughout the commodity and thus not all pests were exposed to the lethal dose, or perhaps some of the pests treated in Step 3 had greater tolerance to the treatment than the pests used in Steps 1 and 2. If the number of survivors is not high, the treatment developer could still submit their data for consideration, or repeat the study at a higher dose.

### Conclusion

The current guidelines for treating wood packaging material under ISPM No. 15 have apparently reduced the numbers of wood pests present in international trade (Haack & Petrice, 2009). However, the current schedules for heat treatment and methyl bromide fumigation (IPPC, 2009a) were subjected to less stringent testing than what is proposed now under the 2010 draft Appendix (Smith, 1991, 1992; IPPC, 2010). We encourage development of new phytosanitary treatments for inclusion in ISPM No. 15. However, we caution against requiring probit 9 efficacy for approval because it places a burden on treatment developers, is not biologically justifiable for most wood pests, and currently allows for pseudoreplication rather than focusing on statistical reliability. Moreover, requiring probit 9 for wood pests could inhibit new treatment development, ensuring continued dependence on less well tested treatments now approved under ISPM 15. As an alternative, we suggest a 3-step process for treatment development that has high statistical reliability. We recognize that the studies proposed in Steps 1–3 will take place in laboratories, therefore any phytosanitary treatments developed using these protocols and later approved for inclusion in ISPM No. 15 should be modified as needed based on subsequent real-world experience.

**Table 2** Relationship between sample size and statistical reliability expressed as 95% confidence

| Sample size | Reliability* |
|-------------|--------------|
| 1           | 0.050000     |
| 5           | 0.549280     |
| 10          | 0.741134     |
| 20          | 0.860892     |
| 30          | 0.904966     |
| 40          | 0.927842     |
| 50          | 0.941845     |
| 60          | 0.951297     |
| 100         | 0.970487     |
| 299         | 0.990031     |
| 2995        | 0.999000     |
| 93 613      | 0.999968     |

\*Values based on the formula  $n = \log(1-C)/\log(r)$  where  $n$  is the number of individuals tested (sample size);  $C$  is the confidence level (set between 0 and 1; we used 0.95); and  $r$  is the level of reliability (set between 0 and 1; Beyer, 1968).

## Acknowledgements

The authors thank E.A. Allen, K.O. Britton, L.M. Humble, P.I. Morris, T.R. Petrice and J.S. Stanovick for comments on an earlier version of this manuscript, and the members of the International Forest Quarantine Research Group (IFQRG) for many stimulating conversations on the topic of this paper. K.H. thanks USDA, Methyl Bromide Transitions Grant Program for funding (Grant No. 2009-51102-05652) and A.U. thanks National Resources Canada (Canadian Forest Service) for guidance and financial support.

## Chercher des alternatives au probit 9 lors du développement de traitements pour le bois d'emballage dans le cadre de la NIMP No. 15

La Norme internationale pour les mesures phytosanitaires NIMP No. 15 (*Réglementation des matériaux d'emballage en bois dans le commerce international*) présente des recommandations pour traiter le bois d'emballage utilisé dans le commerce international. Elles consistent actuellement en deux traitements phytosanitaires approuvés: le traitement par la chaleur et la fumigation au bromure de méthyle. De nouveaux traitements sont en cours de développement et sont nécessaires étant donné que le bromure de méthyle est supprimé progressivement. L'efficacité Probit 9 (100% de mortalité d'au moins 93613 organismes testés) a été suggérée comme critère d'évaluation pour de nouveaux traitements du bois, et est basée sur la recherche sur les mouches des fruits. Nous nous interrogeons sur la pertinence d'exiger une efficacité probit 9 pour les ravageurs du bois (insectes, nématodes et champignons) et discutons des défis pour atteindre cette exigence. A la place, nous suggérons une approche alternative en 3 étapes au laboratoire. La première étape implique des expérimentations au laboratoire (tri préliminaire) pour estimer la dose létale pour le stade le plus sensible de chaque organisme cible. Nous considérons chaque pièce de bois infestée comme étant une unité expérimentale, et non pas les ravageurs individuels pour éviter des pseudorépétitions. L'étape 2 demande des expérimentations répétées (avec aucun survivant) à la dose létale estimée. Nous suggérons une taille minimale d'échantillon de 60 unités expérimentales, ce qui permet d'atteindre une fiabilité statistique de 0.95 avec un niveau de confiance de 95%. La troisième étape implique des études en conditions opérationnelles simulées en utilisant des échantillons de bois similaires en taille au bois d'emballage et infestés à des niveaux qui reflètent les conditions sur le terrain.

## Поиск альтернатив критерию оценки пробит-9 при разработке обработок древесных упаковочных материалов в соответствии с МСФМ № 15

В Международном стандарте по фитосанитарным мерам (МСФМ) № 15 (Регулирование древесных упаковочных материалов в международной торговле) представлено

руководство по обработке древесных упаковочных материалов, используемых в международной торговле. В настоящее время существуют две одобренных фитосанитарных обработки: тепловая обработка и фумигация с помощью бромистого метила. Разрабатываются также новые виды обработок, потребность в которых возникает в связи с постепенным сокращением использования бромистого метила. Эффективность пробит-9 (100%-ая смертность не менее чем 93613 тестируемых организмов), основанная на исследовании плодовых мух, была предложена в качестве критерия оценки новых обработок древесины. Авторы подвергают сомнению требование эффективности пробит-9 для вредителей древесины (насекомых, нематод и грибов) и рассматривают проблемы, связанные с этим требованием. Вместо этого они предлагают внедрить 3-ступенчатый лабораторный альтернативный подход. На ступени 1 производятся лабораторные эксперименты (скрининг), позволяющие дать оценку летальной дозы для наиболее толерантной стадии каждого вредного организма-мишени. При этом, чтобы избежать псевдорепликации, авторы исходят из того, что экспериментальной единицей является каждый зараженный кусок древесины, а не отдельные вредные организмы. Ступень 2 требует повторных экспериментов (без выживших особей) при выявленной летальной дозе. Предлагается минимальный размер выборки в 60 экспериментальных единиц, который позволяет достигать 0,95 статистической надежности при 95%-ом уровне достоверности. Ступень 3 требует исследования в симулируемых полевых условиях с использованием при этом образцов древесины, сходных по размеру с упаковочной и зараженных до уровней, которые отражают полевые условия.

## References

- Amett RH (2000) *American Insects: A Handbook of the Insects of America North of Mexico*. 2nd edn. CRC Press, Boca Raton, FL (US).
- Amett RH, Thomas MC, Skelley PE & Frank JH, eds (2002) *American Beetles. Vol. 2. Polyphaga: Scarabaeoidea through Curculionoidea*. CRC Press, Boca Raton, FL (US).
- Baker AC (1939) The basis for treatment of products where fruit flies are involved as a condition for entry into the United States. US Department of Agriculture Circular 551.
- Baker RT, Cowley JM, Harte DS & Frampton ER (1990) Development of a maximum pest limit for fruit flies (Diptera: Tephritidae) in produce imported into New Zealand. *Journal of Economic Entomology* **83**, 13–17.
- Beyer WH (ed.) (1968) *CRC Handbook of Tables for Probability and Statistics*. Chemical Rubber Company, Cleveland, OH (US).
- Bliss CI (1934) The method of probits. *Science* **79**, 38–39.
- Brockerhoff EG, Bain J, Kimberley MO & Knížek M (2006) Interception frequency of exotic bark and ambrosia beetles (Coleoptera: Scolytinae) and relationship with establishment in New Zealand and world-wide. *Canadian Journal of Forest Research* **36**, 289–298.
- Callan BE & Carris LM (2004) Fungi on living plant substrata, including fruits. In *Biodiversity of Fungi. Inventory and Monitoring Methods* (Eds Mueller GM, Bills GF & Foster MS), pp. 105–126. Elsevier, Academic Press, London (UK).

- Cocquemot C & Lindelöw A (2010) Longhorn beetles (Coleoptera, Cerambycidae). *BioRisk* **4**, 193–218.
- Couey HM & Chew V (1986) Confidence limits and sample size in quarantine research. *Journal of Economic Entomology* **79**, 887–890.
- Denux O & Zagatti P (2010) Coleoptera families other than Cerambycidae, Curculionidae *sensu lato*, Chrysomelidae *sensu lato* and Coccinellidae. *BioRisk* **4**, 315–406.
- Eichlin TD & Duckworth WD (1988) *The Moths of America North of Mexico, Sesioidea, Sesidae, Fascicle 5.1*. Wedge Entomological Research Foundation, Washington, DC (US).
- Fauna Europaea (2010) Fauna Europaea. <http://www.faunaeur.org/> [accessed on 27 November 2010].
- Finney DJ (1971) *Probit Analysis*, 3rd edn. Cambridge University Press, Cambridge (UK).
- Follett PA & McQuate GT (2001) Accelerated development of quarantine treatments for insects on poor hosts. *Journal of Economic Entomology* **94**, 1005–1011.
- Follett PA & Neven LG (2006) Current trends in quarantine entomology. *Annual Review of Entomology* **51**, 359–385.
- Haack RA (2006) Exotic bark and wood-boring Coleoptera in the United States: recent establishments and interceptions. *Canadian Journal of Forest Research* **36**, 269–288.
- Haack RA & Petrice TR (2009) Bark- and wood-borer colonization of logs and lumber after heat treatment to ISPM 15 specifications: the role of residual bark. *Journal of Economic Entomology* **102**, 1075–1084.
- Haack RA & Slansky F (1987) Nutritional ecology of wood-feeding Coleoptera, Lepidoptera, and Hymenoptera. In *Nutritional ecology of insects, mites, and spiders* (Eds Slansky F & Rodriguez JG), pp. 449–486. John Wiley, New York, NY (US).
- Haack RA, Hérard F, Sun J & Turgeon JJ (2010) Managing invasive populations of Asian longhorned beetle and citrus longhorned beetle: a worldwide perspective. *Annual Review of Entomology* **55**, 521–546.
- Hanks LM (1999) Influence of the larval host plant on reproductive strategies of cerambycid beetles. *Annual Review of Entomology* **44**, 483–505.
- Heppner JB (2008) Butterflies and moths (Lepidoptera). In *Encyclopedia of Entomology*, 2nd edn (Ed., Capinera JL), pp. 626–672. Springer, Dordrecht (NL).
- Hoover K, Uzunovic A, Gething B, Dale A, Leung K, Ostiguy N & Janowiak JJ (2010) Lethal temperature for pinewood nematode, *Bursaphelenchus xylophilus*, in infested wood using microwave energy. *Journal of Nematology* **42**, 101–110.
- (IPPC) International Plant Protection Convention (2002) *International Standards for Phytosanitary Measures: Guidelines for Regulating Wood Packaging Material in International Trade, Publication No. 15*. Food and Agriculture Organization of the United Nations, Rome (IT).
- (IPPC) International Plant Protection Convention (2009a) *International Standards for Phytosanitary Measures: Revision of ISPM No. 15, Regulation of Wood Packaging Material in International Trade*. Food and Agriculture Organization of the United Nations, Rome (IT).
- (IPPC) International Plant Protection Convention (2009b) *International Standards for Phytosanitary Measures: Phytosanitary Treatments for Regulated Pests, Publ. No. 28*. Food and Agriculture Organization of the United Nations, Rome (IT).
- (IPPC) International Plant Protection Convention (2010) *Draft Appendix to ISPM 15: 2009 Submission of New Treatments for Inclusion in ISPM 15*. Food and Agriculture Organization of the United Nations, Rome (IT).
- Landolt PJ, Chambers DL & Chew V (1984) Alternative to the use of probit 9 mortality as a criterion for quarantine treatments of fruit fly (Diptera: Tephritidae) infested fruit. *Journal of Economic Entomology* **77**, 285–287.
- LeOra Software (2007) *PoloPlus Probit and Logit Analysis User's Guide, Version 2.0*. LeOra Software, Petaluma, CA (US).
- Lieutier F, Day KR, Battisti A, Grégoire JC & Evans HF (eds) (2004) *Bark and Wood Boring Insects in Living Trees in Europe, a Synthesis*. Kluwer, Dordrecht (NL).
- Liquido NJ, Griffin RL & Vick KW (1997) Quarantine Security for Commodities: Current Approaches and Potential Strategies. US Department of Agriculture, Agricultural Research Service 1996–2004.
- Lopez-Vaamonde C, Agassiz D, Augustin S, De Prins J, De Prins W, Gomboc S, Ivinskis P, Karsholt O, Koutroumpas A, Koutroumpa F, Laštůvka Z, Marabuto E, Olivella E, Przybyłowicz L, Roques A, Ryrholm N, Šefrová H, Šima P, Sims I, Sinev S, Skulev B, Tomov R, Zilli A & Lees D (2010) Lepidoptera. *BioRisk* **4**, 603–668.
- McCullough DG, Work TT, Cavey JF, Liebhold AM & Marshall D (2006) Interceptions of nonindigenous plant pests at US ports of entry and border crossings over a 17-year period. *Biological Invasions* **8**, 611–630.
- Poole RW & Gentili P (eds) (1996) *Nomina Insecta Nearctica: A Check List of the Insects of North America. Vol. 1: Coleoptera, Strepsiptera*. Entomological Information Services, Rockville, MD (US).
- Rasplus JY, Villemant C, Paiva MR, Delvare G & Roques A (2010) Hymenoptera. *BioRisk* **4**, 669–776.
- Robertson JL, Russell RM, Preisler HK & Savin E (2007) *Bioassays with Arthropods*, 2nd edn. CRC Press, Boca Raton, FL (US).
- Roques A, Rabitsch W, Rasplus JY, Lopez-Vaamonde C, Nentwig W & Kenis M (2009) Alien terrestrial invertebrates of Europe. In *Handbook of Alien Species in Europe* (ed. DAISIE), pp. 63–79. Springer, Dordrecht (NL).
- Roth H (1989) Concepts and recent developments in regulatory treatments. In *Plant Protection and Quarantine*, vol. 3 (ed. Kahn RP), pp. 117–144. CRC Press, Boca Raton, FL.
- Ryss A, Vieira P, Mota M & Kulinich O (2005) A synopsis of the genus *Bursaphelenchus* Fuchs, 1937 (Aphelenchida: Parasitaphelenchidae) with keys to species. *Nematology* **7**, 393–458.
- Sauvard D, Branco M, Lakatos F, Faccoli M & Kirkendall LR (2010) Weevils and bark beetles (Coleoptera, Curculionidae). *BioRisk* **4**, 219–266.
- Schiff NM, Valley SA, LaBonte JR & Smith DR (2006) *Guide to the Siricid Woodwasps of North America. FHTET-2006-15*. U.S. Department of Agriculture, Forest Service, Forest Health Technology Enterprise Team, Morgantown, WV (US).
- Smith RS (ed.) (1991) *The Use of Heat Treatment in the Eradication of the Pinewood Nematode and its Vectors in Softwood Lumber*. Report of the Task Force on Pasteurization of Softwood Lumber. Forintek Canada Corporation, Vancouver, BC (Canada).
- Smith RS (1992) Eradication of pinewood nematodes in softwood lumber. *Proceedings of the Canadian Wood Preservation Association* **13**, 185–206.
- Unterseher M, Schnittler M, Dormann C & Sickert A (2008) Application of species richness estimators for the assessment of fungal diversity. *FEMS Microbiology Letters* **282**, 205–213.
- USDA APHIS (US Department of Agriculture, Animal and Plant Health Inspection Service) (2010) Treatment manual. <https://manuals.cphst.org/TIndex/index.cfm> [accessed on 27 November 2010].
- (TPFQ) Technical Panel on Forest Quarantine (2008) Report technical panel on forest quarantine meeting, Moscow, Russia, 02-06 July 2007. [https://www.ippc.int/file\\_uploaded/1206005845183\\_Report\\_TPFQ\\_2007\\_FINAL\\_2008\\_03\\_17.doc](https://www.ippc.int/file_uploaded/1206005845183_Report_TPFQ_2007_FINAL_2008_03_17.doc) [accessed on 27 November 2010].
- Wood SL & Bright DE (1992) A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: taxonomic index. *The Great Basin Naturalist Memoirs* **13**, 1–1553.
- Zahid MI, Grgurinovic CA & Walsh DJ (2008) Quarantine risks associated with solid wood packaging materials receiving ISPM 15 treatments. *Australian Forestry* **71**, 287–293.