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FORUM

Appropriateness of Probit-9 in the Development of Quarantine Treatments for Timber and Timber Commodities

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ABSTRACT Following the increasing international phasing out of methyl bromide for quarantine purposes, the development of alternative treatments for timber pests becomes imperative. The international accreditation of new quarantine treatments requires verification standards that give confidence in the effectiveness of a treatment. Probit-9 mortality is a standard for treatment effectiveness that has its origin in fruit fly research, and has been adopted by the United States Department of Agriculture for fruit flies and several other pests. Following this, the probit-9 standard has been adopted as a benchmark for many quarantine treatments worldwide. This article discusses aspects of the application of this concept for a range of timber pests. Problematic issues include the often small pest populations available for testing, the limits of modeling pest responses to a treatment in the absence of sufficient numbers for treatment verification, the species diversity of pests and host materials and the physical and chemical conditions of host material or treatment conditions. Where treatment verification by killing large numbers of individuals is impossible, data collected from small populations or under specific conditions must be interpreted with caution. We discuss possible alternative approaches to probit-9 as a treatment efficacy standard.

KEY WORDS International Standard for Phytosanitary Measures No. 15, International Plant Protection Convention, quarantine treatments

Wood packaging material (WPM) such as crating, dunnage, and pallets used in international trade is recognized as a major pathway by which bark- and wood-infesting organisms can be moved between countries (Allen and Humble 2002, Haack 2006, McCullough et al. 2006, Zahid et al. 2008, Roques et al. 2009, Haack et al. 2010a). WPM is commonly of low commercial value and therefore often of poor quality. Wood for WPM may be sourced from by-products of milling or salvaged from trees killed by wildfires or insect outbreaks. Potential pests may be present in wood used for WPM at the time of harvest or colonize the wood after harvest (Zahid et al. 2008, Haack and Petrice 2009). Thus, the prevalence of quarantine pests and pathogens in WPM may far exceed that in quality timber or export quality horticultural commodities.

In 2002, the International Plant Protection Convention published the International Standard for Phytosanitary Measures No. 15: Guidelines for regulating wood packaging material in international trade (ISPM No. 15) (IPPC 2002). The standard was modified in 2006 (IPPC 2006), and a further revision was published in 2009 (IPPC 2009a). 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 WPM (IPPC 2002, 2006). In 2009, the wording was changed to "reduce significantly the risk of introduction and spread of most quarantine pests" (IPPC 2009a). ISPM No. 15 recognizes two measures to adequately treat WPM: heat treatment at a minimum temperature of 56°C for at least 30 min throughout the profile of the wood, including the core (often abbreviated to "56/30"), and methyl bromide fumigation (of which the conditions have changed over consecutive versions of the standard). The 2009 revision added the use of debarked wood as a requirement with a specified tolerance (IPPC 2009a). The Draft appendix to ISPM 15:2009 (IPPC 2010) on test criteria for new treatments for the first time included fungi and oomycetes as pests of concern.

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The pest groups that need to be managed by these WPM treatments include various beetle (Coleoptera) families (Anobiidae, Bostrichidae, Buprestidae, Cerambycidae, Curculionidae, Lyctidae, Oedemeridae, Scolytinae [formerly Scolytidae]); Siricidae (Hymenoptera); Isoptera; and the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) (IPPC 2002, 2006). Recent research on treatment efficacy against organisms, such as the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), has raised some discussion over the heat-treatment schedules prescribed by ISPM No. 15 (McCullough et al. 2007, Myers et al. 2009, Goebel et al. 2010, Haack and Petrice 2010). Also, a considerable variety of wood fungi has been found to be resistant to temperatures of 56°C or higher (Newbill and Morell 1991, Schmidt 2007, Uzunovic and Khadempour 2007, Ramsfield et al. 2010). Methyl bromide fumigation at the rates required by ISPM No. 15 will not be effective against many fungi, such as *Ceratocystis fagacearum* (Bretz) J. Hunt (Tubajika and Barak 2007).

The first two versions of ISPM No. 15 also mentioned other treatments that were being considered and that may be approved when appropriate data become available, such as fumigation with phosphine, sulfuryl fluoride or carbonyl sulfide, chemical pressure impregnation, and irradiation. Currently, there is no international standard for quarantine treatment of logs, lumber, fuelwood, or wood chips, and international trade in these commodities is not subject to internationally agreed risk minimization measures, although they may have significant pest loads (Kliejunas et al. 2003, Trott and Lum 2009, Haack et al. 2010b).

Since the Montreal Protocol (UNEP 2006) came into force in 1989, there has been an international effort to reduce and phase out the use of methyl bromide because of its ozone layer-depleting properties (although the protocol allows use of methyl bromide for quarantine purposes). Finding alternatives for the use of methyl bromide in quarantine has become increasingly important. In 2008, the European Union banned the use of methyl bromide for most purposes, including quarantine treatments as of March 2010 (Commission of the European Communities 2008), making the accreditation of alternative quarantine treatments imperative.

Treatment of quarantine pests with methyl bromide has been historically accepted by many countries and for a wide range of pests. However, the acceptance of novel quarantine treatments usually relies on the presence of efficacy data for these treatments. As described in ISPM No. 28 (IPPC 2009b), the acceptance of new treatments, including alternative treatments for WPM or other wood products for an international standard by the Standards Committee of the International Plant Protection Convention (IPPC) would require an agreement on a required standard of efficacy data by the Technical Panel on Phytosanitary Treatments (TPPT). This standard would then need to be addressed by researchers aiming to demonstrate the efficacy of a given treatment. In international plant quarantine, probit-9 mortality of a pest is often seen as the benchmark for the efficacy of phytosanitary treatments and has in the past been required by the TPPT. Also, probit-9 was suggested as the required efficacy in the Draft appendix to ISPM 15:2009 (IPPC 2010) for the beetle families which were listed in the first two versions of ISPM No. 15. Notably, neither of the currently approved treatments for ISPM No. 15 has been demonstrated to meet this standard for wood-infesting insects or fungi. The purpose of this article is to discuss some of the current issues regarding efficacy testing for phytosanitary treatments of timber pests and the need for an alternative approach to probit-9 as an efficacy standard.

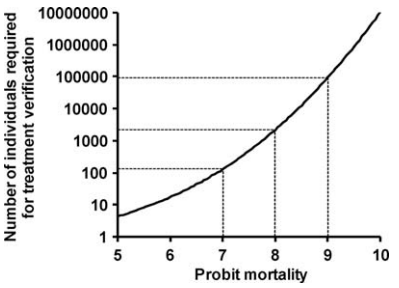

Fig. 1. Number of individuals required to be tested without survivors to achieve 95% confidence (calculated from Couey and Chew 1986) as a function of the required mortality in a treatment verification experiment (expressed in probits).

Limitations of Probit-9 as a General Treatment Efficacy Standard

For horticultural commodities, the United States has for many decades applied a standard of probit-9 efficacy for the treatment of fruit flies (Diptera: Tephritidae) or pests of similar significance. Probit-9 efficacy requires 99.9968329% mortality of a pest (often rounded to 99.9968%) after a treatment. Couey and Chew (1986) provided equations to determine the number of insects that would have to be tested with no survivors for a given treatment, for a given mortality, and a given level of confidence. The relationship between the targeted mortality and the required number of test individuals is exponentially shaped, i.e., for a relatively small increase in mortality, a large additional number of individuals may be needed for testing (Fig. 1). If the conventional confidence limit of 95% is applied, probit-9 efficacy (99.9968329%) would require no survivors in a minimum of 94,588 insects tested. However, for a mortality rate rounded down to 99.9968%, it would require no survivors in a minimum of 93,616 insects tested. This number or approximate numbers have often been reported in the literature (Couey and Chew 1986, Follett and Neven 2006). Note that Couey and Chew (1986) and many subsequent papers specify a number of 93,613 test individuals, a deviation due to a rounding error. Table 1 gives some examples of the number of test individuals required for a range of probit or percentage mortalities.

Table 1. Number of test individuals required (calculated from Couey and Chew 1986) in treatment efficacy trials to achieve a range of mortality levels and their associated probit values, at 95% confidence level

Mortality (%)	Probit	No. test individuals required
97.72499	7	131
99	7.3263	299
99.86501	8	2,218
99.9	8.0902	2,995
99.99	8.7190	29,956
99.9968	8.9976	93,616
99.99683	9	94,587
99.999	9.2649	299,572

The concept of probit-9 as an efficacy standard originated with Baker (1939), who used it to recommend cold and heat treatments for fruit flies, such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and melon fly, (*Bactrocera cucurbitae* (Coquillett). Baker (1939) used a probit transformation to describe the relationship between the duration of a given treatment and the mortality of the targeted pest. The concept of probit analysis to analyze dose–mortality responses was first published by Bliss (1934, 1935) for populations of organisms whose tolerance to the applied dose of a toxin follows a log-normal distribution, and where probit-5 designates 50% mortality. Baker (1939) defined the mortality level of probit-9 as a satisfactory level of quarantine security and read the required treatment time along a regression line between the logarithm of treatment duration and probit mortality. He provided no rationale for selecting probit-9 mortality as an efficacy criterion other than to "assure no survival of [fruit fly] eggs or larvae in the products treated." Importantly, Baker (1939) did not test sufficient individuals to confirm no survivors among >93,616 insects at the dose expected to achieve probit-9 mortality. Although not all dose–response data may be distributed log-normally, probit-9 as an efficacy standard does not require probit analysis, but merely a function that gives a mortal dose to kill 99.9968% of a population. Logit, complementary log-log, or Gompertz transformations may give a better fit to some data sets (Hallman 1993, Liquido et al. 1997, Follett and Neven 2006).

Following Baker (1939), the use of the probit-9 standard for fruit flies and other significant pests by the U.S. Department of Agriculture (USDA) has come to require treatment verification demonstrating efficacy at the 95% confidence level, i.e., using 93,616 insects, or even greater numbers when considering control mortalities in untreated insect populations (Follett and Neven 2006). The arbitrary nature of this standard has been acknowledged, including by USDA workers (Landolt et al. 1984, Vail et al. 1993, Liquido et al. 1997). Landolt et al. (1984), Vail et al. (1993), Follett and McQuate (2001), and Hansen and Johnson (2007) found that the probit-9 standard may be too stringent for rarely infested commodities or poor hosts.

The probit-9 standard required by the United States generated extensive treatment verification experiments for many fruit fly (Tephritidae) pests and a wide range of treatments and treated commodities (Table 2). In all listed examples in Table 2, researchers exceeded (if not by much) the required 93,616 tested individuals to achieve official acceptance of the treatment as a quarantine treatment in the United States. It is important to note that for fruit flies as well as other pests, probit-9 efficacy has usually been shown by demonstrating mortality for a high number of individuals, but not necessarily a high number of independent experimental units. For example, one piece of fruit or timber used in testing usually hosts multiple individuals, which would not be statistically independent. One treatment application will usually affect multiple pieces of fruit or timber, which again would not be statistically independent from each other. In the ecological literature, the use of inferential statistics where treatments are not replicated (though samples may be) or where replicates are not statistically independent, has been coined "pseudoreplication" (Hurlbert 1984). To some extent, this criticism could be extended to mortality experiments in toxicology and quarantine treatment research. However, conducting treatment efficacy trials to the most rigorous statistical standards and avoiding pseudoreplication entirely would be largely beyond the resources available for this type of work.

With the exception of the *B. xylophilus* (e.g., Hoover et al. 2010), we are not aware of treatment verification experiments to probit-9 standards for nontephritid pests. Although few other pests in international quarantine policy have gained the notoriety of fruit flies, an important reason for the absence of successful verification trials for such pests may be the difficulty of acquiring enough test individuals. Table 3 gives some examples of numbers of tested individuals in treatment efficacy tests for a range of coleopteran, hemipteran and lepidopteran pests in horticulture and forestry. It is notable that often larger numbers of individuals have been used in trials using horticultural commodities or artificial laboratory diets, whereas trials on timber pests have commonly used comparatively few individuals, largely for practical reasons.

Australia, Japan, and New Zealand accept a quarantine treatment efficacy of 99.99% for many pests and commodities (Follett and Neven 2006). At a 95% confidence level, this translates to 29,956, i.e., ≈30,000 individuals tested (Couey and Chew 1986).

In the United States, suggestions for alternatives to a stand-alone probit-9 mortality standard have included considerations of the likelihood of mating pairs of insects surviving in a single consignment (Landolt et al. 1984), low pest prevalence on poor hosts (Follett and McQuate 2001), or quantitative pathway analyses to model numbers of pests arriving in susceptible areas (Hennessey 2004). The concept of "maximum pest limit," developed in New Zealand (Baker et al. 1990, Cowley et al. 1993), which adds considerations of consignment size and pest prevalence to treatment efficacy, also was adopted in the United States by

Table 2. Summary data for quarantine treatments verification experiments effective for various fruit fly (Diptera: Tephritidae) species in which >93,616 individual insects were treated in at least one treatment

Tephritid species	Commodity	Treatment	Reference
Anastrepha ludens (Loew)	Grapefruit, mangoes	Hot air, hot water, irradiation	Sharp 1988, Sharp et al. 1989b, Mangan and Ingle 1994, Hallman and Martinez 2001, Bustos et al. 2004
Anastrepha obliqua (Macquart)	Mangoes	Hot air, hot water, irradiation	Sharp 1988; Sharp et al. 1988, 1989b; Sharp and Picho-Martinezi 1990; Mangan and Ingle 1992; Bustos et al. 2004
Anastrepha serpentina (Wiedemann)	Mangoes	Hot water, irradiation	Sharp 1988, Sharp et al. 1989c, Bustos et al. 2004
Anastrepha suspensa (Loew)	Carambolas, grapefruit, mangoes, oranges, guavas	Hot air, hot water, vapor heat, irradiation, cold storage, methyl bromide	Sharp 1988; Sharp et al. 1988, 1989a; Gould and Sharp 1990; Hallman 1990; Hallman and Sharp 1990; Hallman et al. 1990; von Windeguth and Gould 1990; Gould and von Windeguth 1991; Gould and Sharp 1992; Hallman and King 1992; Sharp 1992, 1993; Sharp and Hallman 1992; Sharp and McGuire 1996
Bactrocera cucumis (French)	Zucchini	Vapor heat	Corcoran et al. 1993
Bactrocera cucurbitae (Coquilett)	Bananas, bell peppers, carambolas, cucumbers, eggplants, lychees, papayas	Hot air, hot water, irradiation, cold storage, methyl bromide	Seo et al. 1973, Armstrong 1982, Armstrong and Garcia 1985, Armstrong et al. 1989, 1995a,b; Follett and Armstrong 2004, Armstrong and Follett 2007
Bactrocera dorsalis (Hendel)	Bananas, carambolas, cucumbers, lychees, papayas	Hot air, hot water, vapor heat, irradiation, cold storage, methyl bromide	Seo et al. 1973, 1974a; Armstrong 1982; Armstrong and Garcia 1985; Armstrong et al. 1989, 1995a,b; Armstrong and Follett 2007
Bactrocera jarvisi (Tryon)	Mangoes	Irradiation	Heather et al. 1991
Bactrocera tryoni (Froggatt)	Avocados, blueberries, grapes, lemons, mandarins, mangoes, oranges	Hot air, hot water, irradiation,	Heather et al. 1991, 1996, 1997; Jessup 1992, 1994; Jessup et al. 1993, 1998; de Lima et al. 2007
Ceratitis capitata (Wiedemann)	Apricots, bananas, carambolas, lemons, lychees, mandarins, mangoes, nectarines, oranges, papayas, peaches, plums, strawberries	Hot air, hot water, irradiation, cold storage, methyl bromide	Armstrong 1982; Spitler and Couey 1983; Armstrong and Couey 1984; Armstrong et al. 1984, 1989, 1995a,b; Sharp 1988; Sharp et al. 1989c; Sharp and Picho-Martinezi 1990; Heather et al. 1997, Bustos et al. 2004, Armstrong and Follett 2007, de Lima et al. 2007, Torres-Rivera and Hallman 2007
Rhagoletis mendax Curran	Blueberries	Irradiation	Sharp and Polavarapu 1999

Mangan et al. (1997). In all of these cases, the pests considered were fruit flies. These approaches have improved the simple application of a stand-alone treatment by focusing on the survival and establishment of a pest in a consignment rather than a percentage of mortality. Similar considerations have led to the concept of "systems approaches" to achieve an agreed level of quarantine security for a commodity (Jang and Moffitt 1994). However, the required information or assumptions of pest prevalence have usually restricted these approaches to single pests and single commodities.

Application of Probit-9 Efficacy in Treatments for Timber Pests: Invertebrates

Wood products (including WPM) can harbor a multitude of pests, pathogens, and decay organisms (Haack 2001, 2006; Mireku and Simpson 2002; Brockerhoff et al. 2003, 2006; Gu et al. 2006; Brockerhoff 2009; Li et al. 2009), and an international standard such as ISPM No. 15 needs to account for this large variety as well as the fact that pest diversity can vary markedly between countries of origin and species of timber. This means that treatments of wood products often need to target multiple pests instead of a discrete pest species or genus.

In addition, the nature of populations of many timber pests and the difficulty of rearing a sufficient number of individuals in a laboratory make it impractical to demonstrate probit-9 treatment efficacy on >90,000 individuals for many pests, including cerambycid and buprestid beetles (Barak et al. 2006, 2010).

In horticultural applications, mass rearing of fruit flies is common, either for the development of quarantine treatments or the release of sterile insects (Leppla 1989, Vargas 1989). This is made possible by relatively short generation times from egg to egg of between one and two months, relatively high numbers of eggs produced per female (typically >100) and low mortality of developing insects (for examples, see Liedo and Carey 1994, Clare 1997, Jaldo et al. 2001). Mass-rearing of insect pests of timber, in contrast, has proven more difficult. For example, laboratory rearing of Asian longhorned beetle, *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) takes at least 6 mo per generation, or >1 yr where a diapause-inducing chill period is required, and only ≈50 eggs are typically produced during the lifetime of a female beetle (Dubois et al. 2002, Keena 2002). Treatment

Table 3. Verification experiments for quarantine treatments effective against nontephritid insect pests

Species (order: family)	Commodity/medium	Treatment	No insects tested	Reference
(Coleoptera: Brentidae)				
Cylas formicarius (F.)	Sweet potatoes	Irradiation	15,674 adults	Sharp 1995
Cylas formicarius elegantulus (Summers)	Sweet potatoes	Irradiation	30,655 adults	Hallman 2001
C. f. elegantulus	Sweet potatoes	Irradiation	60,000 adults	Follett 2006a
(Coleoptera: Buprestidae)				
Agrilus planipennis Fairmaire	Ash wood (*Fraxinus*)	Heat	Up to 50 larvae per treatment	Nzokou et al. 2008
A. planipennis	Ash wood (*Fraxinus*)	Heat	Up to 92 larvae per treatment	Myers et al. 2009
A. planipennis	Ash wood (*Fraxinus*)	Sulfuryl fluoride	Up to 2,457 larvae per treatment	Barak et al. 2010
(Coleoptera: Cerambycidae)				
Anoplophora glabripennis Motschulsky	Poplar wood	Methyl bromide	Up to 812 larvae per treatment	Barak et al. 2005
A. glabripennis	Poplar wood	Sulfuryl fluoride	Up to 781 larvae per treatment	Barak et al. 2006
Chlorophorus annularis (F.)	Bamboo poles	Methyl bromide	Up to 671 larvae per treatment	Barak et al. 2009
C. annularis	Bamboo poles	Sulfuryl fluoride	Up to 479 larvae per treatment	Yu et al. 2010
Tetropium fuscum (F.)	Fresh spruce wood	Heat	Up to 35 larvae, 10 pupae, and 10 adults per treatment	Mushrow et al. 2004
(Coleoptera: Curculionidae)				
Asynonychus cervinus (Boheman)	Lemons	Irradiation	6,500 eggs	Johnson et al. 1990
Conotrachelus nenuphar (Herbst)	Apples	Irradiation	25,000 adults	Hallman 2003
Euscepes postfasciatus (Fairmaire)	Sweet potatoes	Irradiation	62,323 adults	Follett 2006a
Sternochetus mangiferae (F.)	Mangoes	Irradiation	Up to 1,324 beetles (mixture of larvae, pupae and adults) per treatment	Seo et al. 1974b
S. mangiferae	Mangoes	Irradiation	Up to 169 beetles (mixture of larvae, pupae and adults) per treatment	Follett 2001
(Hemiptera: Diaspididae)				
Aspidiotus destructor Signoret	Pumpkins	Irradiation	32,716 adults	Follett 2006b
Pseudaulacaspis cockerelli (Cooley)	*Strelitzia* leaves	Hot water	54,506 eggs, 11,150 adults, 22,622 nymphs and 14,077 crawlers	Hara et al. 1993
Pseudaulacaspis pentagona (Targioni-Tozzetti)	Potatoes	Irradiation	35,424 adults	Follett 2006c
(Hemiptera: Pseudococcidae)				
Maconellicoccus hirsutus (Green)	Peas	Methyl bromide	Up to 1,694 eggs, 10,751 crawlers and 2,732 nymphs per treatment	Zettler et al. 2002
M. hirsutus	Peas	Vapor heat	Up to 4,389 eggs, 1,940 crawlers, 2,789 nymphs and 370 adults per treatment	Follett 2004
Planococcus citri (Risso)	Limes	Hot water	654 immatures and adults	Gould and McGuire 2000
Pseudococcus affinis (Maskell)	Apples	Cold storage	6,403 first-instars, 3,050 second–third instars, 3,028 female adults	Hoy and Whiting 1997
Pseudococcus odermatti Miller & Williams	Limes	Hot water	654 immatures and adults	Gould and McGuire 2000
(Lepidoptera: Crambidae)				
Ostrinia nubilalis (Hbner)	Laboratory diet	Irradiation	34,760 pupae	Hallman and Hellmich 2009
(Lepidoptera: Gelechiidae)				
Sitrotoga cerealella (Olivier)	No medium	Irradiation	15,264 adults	Hallman and Phillips 2008
(Lepidoptera: Pyralidae)				
Omphisa anastomosalis (Guenée)	Sweet potatoes	Irradiation	30,282 pupae	Follett 2006a
Plodia interpunctella (Hbner)	No medium	Irradiation	22,083 adults	Hallman and Phillips 2008
(Lepidoptera: Tortricidae)				
Cryptophlebia illepida (Butler)	Laboratory diet	Irradiation	11,256 late-instar larvae	Follett and Lower 2000
C. illepida	Laboratory diet	Hot water	4,546 late-instar larvae	Follett and Sanxter 2001
Cryptophlebia ombrodelta (Lower)	Laboratory diet	Hot water	4,434 late-instar larvae	Follett and Sanxter 2001
Cydia pomonella (L.)	Nectarines	Methyl bromide	6,187 eggs	Yokoyama et al. 1987
C. pomonella	Apples	Cold storage	35,203 eggs	Moffitt and Burditt 1989
C. pomonella	Apples	Irradiation	921 first-instar, 30,865 second-instar, 24,707 third-instar, 18,817 fourth-instar, and 4,230 fifth-instar larvae	Burditt and Hungate 1989
C. pomonella	Nectarines	Methyl bromide	27,174 eggs	Yokoyama et al. 1990
C. pomonella	Walnuts	Methyl bromide	34,959 fifth-instar larvae	Hartsell et al. 1991

Continued on following page

Table 3. Continued

Species (order: family)	Commodity/medium	Treatment	No insects tested	Reference
C. pomonella	Apples	Controlled atmosphere cold storage	142,021 larvae, including 40,389 fifth-instar larvae (most tolerant life stage)	Toba and Moffitt 1991
C. pomonella	Cherries	Methyl bromide	10,839 third-instar larvae	Moffitt et al. 1992
C. pomonella	Laboratory diet	Irradiation	100,740 fifth-instar larvae	Mansour and Mohamad 2002
C. pomonella	Apples	Irradiation	32,193 fifth-instar larvae	Mansour and Mohamad 2002
Ecdytolopha aurantiana (Lima)	Oranges	Irradiation	901 pupae	Arthur 2002
Grapholita molesta (Busck)	Laboratory diet	Irradiation	58,779 fifth-instar larvae	Hallman 2004

trials for this organism have relied on field-collected insects (Barak et al. 2005, 2006), a task often made even more difficult because it is a quarantined pest in many countries, including the United States. Similarly, experiments on treatments against *A. planipennis* have relied on field-collected material (Nzokou et al. 2008, Myers et al. 2009, Barak et al. 2010).

For many timber pests, there is likely to be little understanding of the requirements necessary to breed and maintain large laboratory colonies. There is also a risk that organisms derived from laboratory culture may not be representative of natural populations. For fruit flies, this has been demonstrated by Hallman (1994, 2007). To obtain natural sources of insects representing a larger genetic and geographical pool in quantities adequate to conduct and confirm efficacy studies is practically impossible for the majority of timber pests.

Case Study: Pest Prevalence and Maximum Pest Limits for *A. glabripennis* and *A. planipennis*

To establish the required efficacy of a treatment for a specific pest, a possible method is the calculation of a maximum pest limit for a worst-case scenario. For a consignment at the upper end of possible consignment sizes, the greatest possible pest prevalence needs to be determined. Also, the minimum size of a founder population of a pest needs to be determined. From these numbers, the population reduction (mortality) required for a successful quarantine treatment can be calculated. From this, it should be possible to specify the number of individuals required to verify the efficacy of a treatment, at an agreed level of statistical confidence, usually 95%.

To demonstrate the application of assumptions of consignment size, pest prevalence, and maximum pest limit in the calculation of necessary treatment efficacies, we used data for two high-profile timber pests: the Asian longhorned beetle (*A. glabripennis*) and the emerald ash borer (*A. planipennis*).

In this example, we consider pallets constructed from timber from trees that are heavily infested with *A. glabripennis* or *A. planipennis.* We assume that every piece of wood used to construct each pallet is infested at a density equal to the average field population that we have encountered. We also consider that every pallet within a single standard 40-foot-long shipping container is infested, and that each shipping container represents a distinct consignment. We realize that these infestation levels would seldom occur in international trade, but we make these assumptions to allow estimation of the maximum pest limit for the two pests. Based on multiple years of field work and rearing experience with trees infested with *A. glabripennis* from Illinois, and trees infested with *A. planipennis* from Michigan, we have calculated average infestation levels of ≈17 *A. glabripennis* or 72 *A. planipennis* per m^2 of bark surface area (R.A.H. et al., unpublished data).

There are several internationally recognized standards for pallet sizes (ISO 2003). A typical Asia pallet, as commonly used in Asia and Oceania, covers 1,100 by 1,100 mm. A pallet consists of three bearers (typically with a cross section of 90 by 50 mm each) and several slats on the upper and lower side (typically 100–140 mm in width and 25 mm in thickness). The wood used in this pallet would have a volume of ≈0.055 m^3 and a total surface area of ≈5 m^2. Pallets used in Europe and the United States, although of different units of measurement (e.g., typically 1219 by 1016 mm [48 by 40 inches] in the United States), have similar wood volumes and surface areas.

Given that both beetle species initially infest the trees near the bark surface, and assuming that the narrowest dimension along the length of each board (i.e., the thickness) was formerly in contact with the bark, then a typical export pallet would have ≈0.52 m^2 of surface area that was formerly in contact with the bark. For example, for a board measuring 1,100 mm in length by 100 mm in width by 25 mm in thickness, we calculated a surface area of 27,500 mm^2 (1,100 mm by 25 mm = 27,500 mm^2). For the sake of this example of a worst-case scenario, we assume that there will be 100% survival of the larvae even if the wood is debarked. Such an assumption is feasible given that *A. glabripennis* larvae reside in wood for much of their larval development, and *A. planipennis* larvae overwinter in the outer sapwood at a depth of ≈1 cm (McCullough et al. 2007). Therefore, using the abovementioned values for infestation levels and pallet surface area, we calculated that a single pallet could contain ≈9 *A. glabripennis* (17 by 0.52 ≈ 9) or 38 *A. planipennis.* Considering 44 pallets per 40-foot-shipping container (assuming double-stacking), these values resulted in estimates of 396 *A. glabripennis* and

1,672 *A. planipennis* per container. Assuming that a new pest population could become established from a single pair of *A. glabripennis* or *A. planipennis*, any quarantine treatment should allow for no more than one survivor per container (i.e., the maximum pest limit is one individual per consignment). This level of mortality would represent a treatment efficacy of 99.75% (i.e., 395/396) for *A. glabripennis* and 99.94% (1,671/1,672) for *A. planipennis.* This calculation, for the sake of simplicity, does not account for the probabilities of several survivors in a consignment being of the same sex. For the beetle species discussed here, the sex ratio in the field is close to 1:1 (Bancroft and Smith 2005, Wang et al. 2010). Species with markedly uneven sex ratios or those capable of parthenogenesis would have to be considered differently. The social behavior of insect pests also will affect the likelihood of establishment of a pest. Mathematical models exist to assess the probability of introduction for bisexual, parthenogenetic, gregarious, and solitary pests (Yamamura and Katsumata 1999).

Also, our example has not considered the reduced likelihood of establishment of invasive species where only a few individuals enter a new territory. In sexually reproducing insects, the most likely reason for this Allee effect is the difficulty of finding a mate (Liebhold and Tobin 2008). For example, the relationship between founder population size and failure of population establishment has been described for the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) (Tobin et al. 2009). Although the Allee effect will probably influence the chance of establishment of founder populations for *A. glabripennis* or *A. planipennis*, we have made the assumption in our example of a worst-case scenario that two individuals in the same consignment will be able to locate each other, mate and start a population.

If treatment efficacy has to be demonstrated at the 95% confidence level, then (following Couey and Chew 1986) this would require treatment of 1,185 *A. glabripennis* and 5,008 *A. planipennis* without survivors—a requirement well short of 93,616 for probit-9 efficacy. If a modeling approach was taken to extrapolate dose–response data to the required efficacy, then (assuming a probit function provides the best fit of the data) the doses required to achieve a mortality of probit 7.80 and 8.24, respectively, would have to be determined.

This case study shows how the maximum pest limit approach can be used to identify the number of individuals of a pest species that need to be tested to demonstrate the efficacy of a timber treatment. It should not be seen as an endorsement of specific efficacy requirements for the treatment of these two species. Also, although *A. glabripennis* and *A. planipennis* have been well researched in recent years, and although data on their prevalence are available, using these species as surrogates for other cerambycid or buprestid species must be done with caution. Other species may be significantly more or less prevalent in WPM than these two species and also may be more or less tolerant to a possible quarantine treatment. Although further research may be needed to identify the densities in WPM of pest species in the groups listed in the Draft appendix to ISPM 15:2009 (IPPC 2010), we consider that the maximum-pest-limit approach provides an effective way of linking the biology of pests to the development of treatments.

Alternatives to Treatment Verification With >90,000 Insects: Can Dose–Response Relationships be Extrapolated?

Probit analysis has been applied by researchers assessing the efficacy of fumigation with methyl bromide, sulfuryl fluoride or ethanedinitrile and heat, vacuum technology or irradiation against timber pests, such as various termite (Isoptera) species (Osbrink et al. 1987, Su et al. 1989), *A. glabripennis* (Barak et al. 2005, 2006; Ren et al. 2006; Chen et al. 2008), *A. planipennis* (Chen et al. 2008, Myers et al. 2009), *Prionoplus reticularis* White (Cerambycidae; Lester et al. 2000), and *B. xylophilus* (Hoover et al. 2010). Where treatment levels to achieve probit-9 efficacy were given in the above reports (with the exception of *B. xylophilus*), they were extrapolated from tests using smaller numbers of individuals.

The application of probit analysis (or similar approaches to analyze dose–response relationships by using different data transformations) to determine doses necessary to achieve a particular mortality level for pests, including timber pests, does not generally lead to confidence in the outcomes reported in published studies. Typical problems presented for policy makers by published studies estimating mortality include the following:

1. No evidence of pilot studies before the reported study. Pilot studies are usually necessary to determine the most tolerant life stage of the target pests as well as to provide an indication of dose levels necessary to achieve interpretable effects.
2. No discussion of how the numbers of organisms or the number of treatments, as well as the placement of dose levels, were selected.
3. No discussion of mortality in controls and how this affects modeling.
4. No discussion of the type of distribution selected for the modeling, and of how well the data obtained fit the model.
5. Confidence (fiducial) limits are often reported, but their implications are seldom discussed in dose recommendations.
6. No discussion of how far a model can be meaningfully extrapolated beyond the data range in the analyzed data set.

Pilot studies could have a key role in optimizing selection of appropriate sample sizes (such as indicating if any level of mortality might occur in the controls) and choosing dose levels to achieve a spread of experimental mortalities in the test subjects that best suit the desired predicted mortality level. Monte Carlo simulations were used by Robertson et al. (1984) to examine the effects of dose selection and

sample size on the precision of lethal dose estimates for LD_{50} and LD_{90} with a logit model. There does not seem to be an equivalent paper for $LD_{99.99}$ or $LD_{99.9968}$. Nevertheless, the article by Robertson et al. (1984) is informative as an indication of the number of individuals likely to be necessary and the number and placement of doses in relation to the required predicted mortality.

Many literature sources examined reported mortality levels of 100% at one or more doses in their experiments (Lester et al. 2000, Follett 2004, Mushrow et al. 2004). In most cases, this is an inefficient use of resources, as 100% or 0% mortality provide no useful information for a dose–response model.

Reported analyses of dose–response relationships more often than not produce exceedingly large 95% confidence intervals around the probit-9 estimate (Barak et al. 2005, 2006). These intervals may be several times as high as the predicted value as a result of data variability, a poor fit of the model to the data, or small dose ranges coupled with distant extrapolations. There is little guidance on how to use information provided in confidence limits around the estimate. For example, where is the point where the confidence intervals become so wide as to make the estimate unreliable, or should a highly precautionary approach be taken and the upper confidence limit be adopted as the dose necessary to achieve the desired mortality level?

The Need to Account for Physicochemical Characteristics of Host Materials

In horticultural trade, treatments for internally feeding pests, such as fruit flies, are usually assessed for specific commodities. The commodity-specific assessment is based on the assumption that the physical and chemical traits of the host material may affect the availability of the active agent to the pest, or the physiology and behavior of the pest in response to the agent. For example, the USDA Treatment Manual (USDA 2010) prescribes different schedules for the cold treatment of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), which is known as the Queensland fruit fly in Australia, for oranges (*Citrus* spp.) and cherries (*Prunus* spp.), and different schedules for methyl bromide fumigation of *B. tryoni* in cherries, citrus, or grapes (*Vitis* spp.). A notable exception to the commodity-specific assessment of treatments is irradiation, where the dose required to render a species sterile is accepted across a wide range of commodities (USDA 2010).

Most timber pests are not limited to the surface of the timber. A quarantine treatment therefore must address internally feeding pests and be effective throughout the profile of the timber. Many timber pests are capable of colonizing or infesting more than one species, genus, or family of tree. Wood from within a single species or genus can have a range of moisture contents and densities. No studies seem to have considered whether factors such as diet, conditions associated with insect microhabitat or host tree species have any influence on susceptibility of the pest to a treatment. However, there is enough information to indicate that tree species, wood moisture content and wood density can affect the penetration of fumigants in timber (Zahora and Morrell 1989, Scheffrahn et al. 1992, Ren et al. 1997). Diffusion of fumigants in hydrated timber is markedly reduced compared with dry timber (Scheffrahn et al. 1992). Acceptable treatments for a quarantine pest should be effective across a range of timbers, timbers densities, and timber moistures, but data to support any such treatments are scarce.

Extension of the Use of Probit-9 Efficacy to Noninsect Pests

A technical protocol for the assessment of treatments for *B. xylophilus* was proposed by Magnusson and Schröder (2009). With some care in selection of naturally infested material or by inoculating fresh logs, suitable numbers of nematodes can be obtained both for modeling work and confirmatory studies. Tests on nematode-infested wood have typically worked with nematode densities between 100 and 200 individuals per g of wood (e.g., Soma et al. 2001), and Hoover et al. (2010) worked with nematode densities exceeding 2,000 per g of wood. If probit-9 mortality is accepted as a treatment efficacy standard, as proposed by Magnusson and Schröder (2009), then verification tests for *B. xylophilus* should be easy to perform using <1 kg of wood.

For commodities infested with fruit flies, Mangan et al. (1997) and Powell (2003) have argued that even a treatment achieving probit-9 efficacy can be overwhelmed by high pest prevalence, i.e., that one or more mating pairs could survive such a treatment of a single consignment. Although this is theoretically correct, it is not very likely that commercial shipments of export-quality fruit carry such pest loads that a probit-9 treatment can be indeed overwhelmed. However, the small size and high population density of *B. xylophilus* make it quite feasible that significant numbers in a timber consignment could survive a treatment that achieves mortality at the probit-9 level. As discussed above, a more meaningful treatment efficacy requirement may be derived by considering the actual pest prevalence in the consignment, as well as mitigating effects by other factors. Although these considerations often suggest that probit-9 might be too conservative as a standard for fruit flies, it may be too liberal for some timber pests, such as *B. xylophilus.* This problem also applies to fungal pests, for which spore densities on timber may be so high that a treatment with probit-9 mortality leaves significant numbers of survivors.

However, when assessing treatment standards for *B. xylophilus*, the population biology and ecology of the species need to be taken into account. At the upper end of nematode densities in heavy natural infestations of 20,000 per g of wood (Magnusson and Schröder 2009), >600 nematodes per kg of wood could survive a probit-9 mortality treatment. It is not

clear what quarantine risk is posed by such a density of surviving nematodes. If a quarantine treatment applied against *B. xylophilus* is also effective against its insect vectors, which are cerambycid beetle species in the genus *Monochamus*, then the establishment and spread of any surviving *B. xylophilus* individuals becomes much less likely. If the vector does not survive, a major pathway for the nematode pest to move from infested wood into host trees is blocked. Minor pathways (e.g., through soil in the absence of the insect vector of *B. xylophilus*) may still exist (Halik and Bergdahl 1992), and if a suitable vector species is already present in the area of introduction, the postentry infestation of the commodity with beetles may provide another pathway for the establishment of *B. xylophilus.*

Fungi and fungus-like organisms such as oomycetes represent the most difficult group against which to determine efficacy of a treatment. There is a large species diversity of fungi, and genetic diversity within species, in wood (Rayner and Todd 1979, Breuil 2008). Fungal diversity and abundance in wood are influenced by the species and size (e.g., diameter) of the host material, degree of decomposition, and climatic factors in the place of origin (Küffer et al. 2008). Fungi in sapwood may be different from those in heartwood (Breuil 2008). In early stages of decomposition of sapwood, ophiostomatoid fungi associated with insect vectors may dominate, with basidiomycetes invading later (Kim et al. 2005). In international trade, timber of different species may be present in the same consignment, and consist of both sapwood and heartwood.

Fungi can exist as not only as superficial mycelia, spores, or fruiting structures on the surface of timber but also as various types of hyphae ramifying throughout the wood tissues. More complex hyphal structures (mycelia and rhizomorphs) and fruiting bodies, as well as resistant fungal structures such as chlamydospores and sclerotia, also may be present. Some fungi may be present in an inactive desiccated state awaiting revival under appropriate conditions of moisture. This diversity of morphological and physiological states makes it difficult to determine those most tolerant to a quarantine treatment, and testing may focus on the state most likely to be present in the timber.

The use of probit analysis or any related form of dose–response analysis is uncommon for fungi. For some timber fungi, Ibrahim et al. (1992), Cheng et al. (2008), and Yen and Chang (2008) used probit analysis to assess the antifungal properties of some chemical compounds, by quantifying reductions in mycelial growth versus a control. These analyses were not conducted with a view to achieve mortality of the fungi, and extrapolation of their data to a probit-9 mortality level would be biologically meaningless. USDA (2007) stated in the U.S. Federal Register that "the probit-9 standard applies to treatments for insect pests such as fruit flies, not to the treatment of pathogens." Ramsfield et al. (2010) used a logit relationship between temperature and mortality when heat-treating several timber fungi on wood blocks. Importantly, the tested units in this study were not individuals of the pest, but of the host (i.e., one wood block, replicated six times). Using this approach, which only allows the testing of a moderate number of units, shares the statistical issues discussed above for invertebrate species with small testing populations.

An important difference between fungi and many invertebrate populations used for testing the efficacy of treatments is that laboratory-reared fungal colonies, even if divided into hundreds or thousands of individual units, are often clonal and therefore do not represent the genetic diversity of a sexually reproducing population. In invertebrates, only parthenogenetically reproducing populations represent a similarly uniform genetic composition. Using clonal material may not only misrepresent field reality, where many genetically distinct individuals may colonize the host material, but also removes the independence of the sampling units in a tested population.

The Need for Alternatives to the Probit-9 Efficacy Standard

Although arbitrary in nature, probit-9 treatment efficacy has become a common standard for the treatment of horticultural commodities. Considerations in this paper, however, have shown that probit-9 efficacy for timber pests may be too conservative (e.g., for many beetles), too lenient (e.g., for nematodes), or not appropriate (e.g., for fungi) and that treatment verification to the numbers required for probit-9 efficacy (>93,616) is often unrealistic and not achievable.

If a verified probit-9 efficacy standard is not suitable for setting international standards for timber pests, such as ISPM No. 15, are there alternatives? Are there potential universal treatments such as the currently accepted heat treatments and methyl bromide fumigations that may be considered effective against all pests of concern? Do diverse pest profiles require a combination of treatments, and how can the efficacy of such treatments be verified?

The large diversity of timber pests makes it impossible to verify quarantine treatments for most pests. The use of representative (model) species for some groups of pests has been suggested in the Draft appendix to ISPM 15:2009 (IPPC 2010). A representative species for quarantine purposes would have to be among the species of a pest group that are more tolerant to the treatment in question, and abundant in large enough numbers to allow the experimental testing of treatments. A rationale would have to be provided why a particular species is representative of others. The Draft appendix to ISPM 15:2009 (IPPC 2010) requires that the organisms most resistant to treatment are identified and used to evaluate treatment efficacy. This is impractical with groups such as Scolytinae with ≈6,000 described species or Cerambycidae with ≈20,000 species.

The case study presented above has shown an example of an alternative approach to determine the necessary efficacy of a treatment for a specific pest, or

group of pests. A further necessary step in treatment development is the demonstration that a treatment achieves this efficacy. There may be many cases where the required treatment efficacy, i.e., mortality of a pest, is too high to allow treatment verification at a confidence level of 95% (if not enough individuals of the pest can be collected or reared). In such cases, the dose required to achieve the necessary mortality may have to be extrapolated from dose–response experiments. Many internationally accepted historic quarantine treatments are not supported by original research trials. Acceptance of treatments or dosages of treatments, in new international standards, based on such extrapolation and without treatment verification, would represent a paradigm shift with the national plant protection organizations of many countries. Based on the biology of individual pests, experimental and statistical requirements for the development of a treatment would have to be internationally agreed. An example of how modeling (in this case based on Monte Carlo simulations) can use dose–response data from few replications to generate mortality doses for high mortality requirements is provided by Powell (2002), using data by Newbill and Morell (1991) for the heat-resistant wood fungus *Postia placenta* (Fr.) M.J. Larsen & Lombard.

Conclusions

Following extensive use by the USDA, probit-9 mortality is widely recognized as an efficacy standard for the quarantine treatment of insects, especially fruit flies. The extension of this standard to new quarantine treatments for timber and timber products, however, raises a number of problems. It is arbitrary to set probit-9, or 99.9968% mortality as an efficacy standard, and probit-9 mortality may be too conservative for rare pests, or too liberal for highly abundant pests. Verification trials are often impossible where not enough individuals can be found or reared for testing. Extrapolation from modeling dose–response relationships suffer from increasing uncertainties the further the extrapolated values are from observed data. Efficacy testing needs to account for diverse conditions of the host material. Although the probit-9 concept has been widely used for insect pests, its use for other organisms, such as nematodes or fungi, may be inappropriate. Alternatives to a probit-9 efficacy standard must address the expected prevalence of a pest on the material in question, and a maximum pest limit that can be tolerated. Where treatment verification to appropriate numbers may not be possible, the analysis of carefully designed dose–response experiments may be used to define appropriate treatment dosages. The development of new timber treatments for use in international standards needs to consider these alternatives.

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