

WOOD PROTECTION BY INTERFERING WITH INSECT BEHAVIOR: ADEQUACY OF LABORATORY TEST DESIGNS

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Abstract Standardized test methods by which the efficacies of conventional chemical wood preservatives against insects determine their toxic effects against larvae of wood boring beetles or their feeding prevention by termites. Although alternative control strategies to the application of biocides exist, such as interference with insect behavior during mating or when searching for suitable breeding and feeding sites, their acceptance, too, depends on efficacy evaluation. This is why new test set ups are needed which take behavior modifying control strategies into account. This paper will demonstrate how new laboratory tests must be designed or how existing standards can be altered to reliably interpret insect behavior including its successful manipulation in a standardized format.

Key Words Standardized tests, IPM, efficacy evaluation, wood borers, termites

INTRODUCTION

The need to protect wood in service against biological decay is probably as old as wood is used for tools or as building material (Fansa and Vorlauf, 2007; Radkau, 2007). Strategies of wood preservation have changed over time (Richardson, 1978). Today, wood protection of structural timber against the attack by insects mainly relies on the application of synthetic chemical preservatives with mostly insecticidal effects against the target organisms (Robinson, 1990). In most industrialized countries efficacy of preservatives is evaluated through standard test methods before registration in the market. Classical test standards usually date back several decades. Depending on the target pest species, larvae of different developmental stages and sizes or adult insects are exposed artificially to the insecticide-treated commodity by placing them into or on to the material (Stephan et al., 2006). The achieved mortality usually rates the effectiveness of the tested biocide.

Modern demands on wood protection, however, ask for minimum biocide application. Therefore, alternative control strategies are needed, which either supplement pesticide applications in order to reduce their needed amount or which replace classical chemical pest control in total. Integrated pest management (IPM) is such a strategy successfully practiced in agricultural and urban pest control, which also should be applied for timber protection. IPM focuses on threshold levels and on the combination of physical, biological and chemical pest control measures (Royer et al., 1999). One possible way of non chemical pest insect control is through manipulation of their behavior which results in interruption of reproduction cycles. In order to successfully implement behavior modification strategies, however, two fundamental issues have to be addressed: First, detailed knowledge on a pests biology, especially its behavior e. g. during mating or when colonizing new food sources for feeding or reproduction have to be investigated and understood. If the natural chain of behavioral steps in the insect is correctly analyzed, an interference and manipulation of this behavior or parts of it could prevent or at least minimize the risk of an infestation and thus reduce the needs for wood preservatives. And secondly, new standardized test methods have to be designed to reliably analyze the effect of an undertaken manipulation. Occasionally and ideally classical standards may only have to be slightly altered which would ease their general acceptance.

Two behavioral complexes will be presented in the following. The first example will focus on mating and egg laying behavior of the European house longhorn borer (old house borer) *Hylotrupes bajulus*. The second will deal with foraging behavior of European subterranean termites of the genus *Reticulitermes*.

EUROPEAN HOUSE LONGHORN BORER

Larvae of the European house longhorn borer exclusively feed in coniferous wood. Their occurrence is usually synanthropic in dry and build-in timber. During their several years lasting development from small to full grown

larvae they mainly excavate the sap-wood parts. This feeding behavior was taken in to account when the classical test standards “EN 46: Determination of the preventive action against recently hatched larvae of *Hylotrupes bajulus* (Linnaeus)” and “EN 47: Wood preservatives — Determination of the toxic values against larvae of *Hylotrupes bajulus* (Linnaeus)” were designed. Namely, these European standards specify methods for the determination of the toxic values of wood preservatives only against larvae by evaluating mortality after several weeks of biotests. Possible ovicidal effects of active ingredients cannot be evaluated with these specific test methods. Because of this, EN 46 was slightly changed in that way, that recently hatched larvae were not artificially introduced on to the wood (the classical way), but that females are allowed to actively depositing their eggs on treated wood specimens. In other words, the natural behavior of egg deposition was incorporated into the test. Now, with this new standard “EN 46-2: Wood preservatives - Determination of the preventive action against *Hylotrupes bajulus* (Linnaeus) - Part 2: Ovicidal effect” a determination of biocidal effects of a chemical treatment during embryo development in the egg prior and in addition to a larvaecidal effect on recently hatched larvae is possible.

Recent studies on the mating and breeding site selection behavior of *H. bajulus* have revealed, that males rather than females most likely select new breeding sites. Fröhlich (1994) and especially Fetzkoöther et al. (1995) have demonstrated the existence of a male produced sex pheromone for long range attraction of females. Importantly, virgin females are only attracted to males and the pheromone when calling males are present on pine wood, the future breeding side (Hertel and Plarre, 2000; Plarre and Hertel, 2000). Possible interference with host seeking behavior in males might therefore enable new alternative control strategies against this destructive pest of structural timber. These strategies can include the reduction of attractiveness of breeding sites for males or the repellence of host seeking males (Metcalf and Metcalf, 1975). Attractive wood can be chemically or physically altered either to reduce the emission of attractive odors, to mask these odors or to add potent and long lasting repellents. This man made quality change of susceptible wood to resistant wood must then be tested to recently emerged adult male beetles in special designed behavior assays such as different types of olfactometers. The olfactometers or bioassay arenas have to be size adapted to the test insect. The observed behavior has to be critically analyzed and interpreted through ethograms to avoid misinterpretation of results. Time for individual adaptation of a tested insect to the set up has to be considered. Test should only be run during sensible phases of an insect which might have rhythmic characteristics or depend of a specific physiological state (Baker and Cardé, 1984). Usually several pre-tests have to be conducted to set the most suitable parameters.

In order to test different qualities of wood of coniferous origin for their susceptibility to be chosen as breeding sites by males of the old house borer, males of different ages were tested for readiness to respond to olfactory stimuli. The general activeness of males was also recorded on locomotion-compensators for multiples days to identify times of most locomotive activity and to set these in congruity with future experimental times. In the end a covered dual choice glass- arena of rectangle shape with approx. 50 cm by approx. 25 cm x approx. 10 cm was designed with a roughened bottom surface to ease walking for the test insect. Wooden test specimens of different quality were placed at the far end of the arena separated by a barrier reaching two- thirds into the arena. An air flow passed over each test specimen and excited the arena at the near end, where test insects were introduced. Tested males were virgin, at least 4 days old and conditioned to a 12:12 light cycle. Experiments were started shortly after the offset of the light phase and after an adaption period of 10 min. By setting these standards during a complex bioassay a reliable ranking for the relative attractiveness of differently modified timber was generated (Ueckerdt, 2011). Among the tested qualities “glued laminated pine - kiln-dried and form stable” was most attractive, flowed by “kiln-dried and form stable pine” (not laminated and without glue). Conventional “kiln-dried pine” and “air-dried pine” were less attractive. “Kiln-dried and form stable fir” was almost as unattractive as beech, which is a non host wood for *H. bajulus*. Potential repellents were tested in the same manner; however, none of them had a long lasting effect.

EUROPEAN SUBTERRANEAN TERMITES

Preventive Strategy

The test standards most often applied in Europe to evaluate chemically derived termite resistance to structural timber are “EN 117: Wood preservatives — Determination of toxic values against *Reticulitermes* species (European termites)” and “EN 118: Wood preservatives — Determination of preventive action against *Reticulitermes* species (European termites)”. Both standards are forced feeding tests and efficacy is rated through frass damage on the test specimens. The standards have two main weaknesses. At first, only chemicals with a strong repellent or feeding deterrent effect may fulfill the pass-criteria. Poisonous modes of actions of active ingredients, for which

treated wood needs to be consumed by the termites, are most likely to fail, because little damage to the test specimen already lead to an efficacy failure rating. Secondly, comparisons of lab with field data have shown, that preservatives for termite efficacy according to EN 117 are highly overdosed than actually needed to perform well in practice. The reason for that is, that under natural conditions termites usually have a choice of treated structural timber and naturally occurring dead wood. This choice situation should be incorporated into an appropriate test design (Fougerousse, 1973). However, the behavior of foraging subterranean termites is very complex (Lenz, 2009). Most often not all available food source are simultaneously exploited. Although search patterns for food are not random, food location by chance is common, as is food steadiness when conspecific termites were successfully recruited and when pheromone trails were established (Traniello and Leuthold, 2000; Hertel et al., 2010). Four choice tests with three treated and one untreated wooden test specimen rather than a one on one choice situation would minimize the influence of chance and reduce misinterpretation of evaluated food avoidance, especially when repeatedly only the untreated test specimens are attacked. Furthermore, efficacy criteria must be extended to include mortality data to allow evaluation of toxic but non-repellent effects.

Curative Strategy

Controlling ongoing termite infestations is nowadays carried out with baiting techniques when, for whatever reason, fumigation is not practical (Forschler, 1998; Su and Scheffrahn, 1998). Termite baits usually contain active biocides in sub-lethal concentrations and it is the accumulation of additive dosages that kill the reproductives in a termite colony after the bait material was fed to them by worker termites. The evaluation of a bait should therefore fulfill the following requirements. The bait matrix should be more attractive than any other available food source. The active ingredient in the bait must be non-repellent (Rust and Smith, 1993; Bläske and Hertel, 2001). The bait must be dosed with the active ingredient in a concentration that allows transport of bait material into the colony by workers without killing them immediately.

On the other hand, kill of reproductives should not last too long after trophallaxis of baited material has occurred. The European wood protection standardization committee (CEN TC 38, WG 24) currently designs laboratory tests to evaluate the attractiveness of a bait and to calculate the time lag of active ingredients from consumption - to food transfer - to lethal effects in the recipients (Karr et al., 2004). In these tests recipient termites are not allowed to feed themselves, their only food source is bait material passed on to them through trophallaxis by other colony members which had access to the bait. Individually marking termites, e. g. with a specific dye, enables to follow up this chain of feeding and food transfer behavior.

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