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THE USE OF WOOD PRESERVATIVES FOR PHYTOSANITATION: A PENETRATION AND EMERGANCE STUDY

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Summary

Solid wood packaging materials have been identified as an invasive species pathway. In response the Food and Agriculture Organization of the United Nations has formulated International Standard for Phytosanitary Measure Number 15. This standard recommends heat treatment or methyl bromide fumigation as the only acceptable methods for mitigating the invasive species risk associated with solid wood packaging materials. Neither of these methods provides lasting protection, nor do they allow an importing country to directly verify treatment. As an alternative wood preservative treatment of SWPM would allow for the direct verification of treatment and the presence of these chemicals would provide lasting protection. The ability of water-based wood preservatives to penetrate insect galleries within western redcedar was investigated and a study was conducted to assess the ability of wood boring insect larvae to penetrate a wood preservative treated barrier. The results indicated that a majority of the insect galleries could be penetrated with preservative using pressure treatment, while the barrier study indicated that wood boring insect larvae could penetrate a wood preservative treated shell regardless of its thickness. Further studies of larvae and adult tunneling behavior are planned.

1. Introduction

Wood packaging materials (WPM) are defined by the Food and Agriculture Organization of the United Nations (FAO) in International Standards for Phytosanitary Measures (ISPM) # 5 as wood or wood products (excluding paper products) used in supporting, protecting or carrying a commodity (including dunnage). Wood packaging materials are defined further in ISPM #15 as materials made of raw unprocessed wood, including dunnage, but excluding processed wood packaging materials. These processed wood packaging materials are defined in the standard as any material made by adding glue, heat or pressure to the wood (FAO 2006).

ISPM #15 establishes solid wood packaging material (SWPM) as an invasive species pathway (FAO 2006). SWPM is constructed from low- grade lumber and logs, that often harbor forest pests. In order to reduce the costs of material production SWPM is often not seasoned prior to being deployed into the material handling system. This increases the likelihood that pests established in the wood will be able to survive. Modern shipping vessels have greatly reduced the amount of time required to reach distant ports of call, and shipping containers that facilitate rapid loading and unloading of these ships also protect insects and fungi from the harsh environment encountered in transit. These factors all increase the likelihood that potential pests will survive the journey, resulting in an invasive species introduction (McNamara and Kroeker, 2001; Dobensky *et al*, 2001).

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has recorded the number and types of pest interceptions since the early 1900's at United States (US) ports. Between 1985 and August 2001, the agency made 577,829 interceptions with an average of 36,882 interceptions per year. Of the total number of interceptions between 1985 and 2000, 6,827 were scolytids or other wood boring insects (Haack, 2003). However, the agency currently inspects only two percent of all international shipments. The inability to inspect all shipments has, resulted in a number of high profile species introductions, including the introduction of the Asian long horned beetle (*Anoplophora glabripennis*, ALB). The introduction and resulting extermination effort for this species is an example of how contaminated SWPM can serve as a vehicle for species introductions and it also illustrates how changes in the legal code of the importing country can greatly reduce the threat of future introductions.

The ALB was first introduced into New York and then two years later in Chicago. This beetle poses a major threat to tree species of significant industrial and urban forestry importance. The beetle was imported into the United States in infested SWPM from China. Nearly 59 million dollars have been spent on eradication following the discovery of the ALB (Haugen and Iede, 2001; McNamara and Kroeker, 2001; Dwinell, 2001; and 7 CFR Part 319). An interim rule was also passed requiring heat treatment or fumigation of all SWPM originating or passing through China (USDA APHIS, 2004). The number of Scolytidae interceptions made by APHIS in packaging material originating from China following the introduction of this interim rule greatly decreased, demonstrating the effectiveness of such legislation (Haack, 2003).

Similar results have also been observed in Europe, were the pine wood nematode (*Bursaphelenchus xylophilus*, PWN) and the long horned beetle (*Monochamus* spp) that serves as a vector species, are of great concern because of the devastating effects of this nematode on important softwood species in the region. The Plant Quarantine Service of Finland confirmed that 5% of all SWPM from North America or China that they tested was infested with PWN or its vector species (McNamara and Kroeker, 2001; Dwinell and Nickle, 1989). In response to the outbreak, the European Union (EU) passed an emergency measure requiring that all coniferous SWPM be heated, fumigated, or chemical treated. They also required that treated SWPM be marked to indicate compliance with the measure. This ruling went into effect in October of 2001 (USDA APHIS, 2003). No additional outbreaks of PWN have been recorded since the introduction of this legislation. The successful exclusion of both of these species using regulatory approaches led other nations to regulate the importation of non-manufactured wood products. The issue also resulted in the drafting of ISPM #15 in 2002.

ISPM #15 mandates that SWPM must be heat treated (HT) or fumigated using methyl bromide (MB) prior to use in international trade. The material must also be marked to signify that it fulfills the standards outlined in ISPM #15. The guidelines outlined by the International Plant Protection Convention (IPPC) also allow the use of other methods to sanitize SWPM once they have been scientifically proven to be effective (FAO, 2006). Since 2002, when all FAO members agreed to these guidelines, these regulations have been incorporated in the legal codes of numerous countries.

Heat treatment is based on the pasteurization process developed by Louis Pasteur in 1865. This method was adapted by Chidester in the 1930s for sterilization of large wooden members prior to or during treatment with wood preservatives. Chidester (1939) reported that the temperature needed to kill fungi was highly time dependent and concluded that a core temperature of 76°C was adequate if maintained for at least 30 minutes. Chidester found an inverse relationship between time and temperature and concluded that internal temperatures lower than 65°C were impractical because of the long times required to kill fungi (Chidester, 1939).

In 2001, the EU implemented a HT standard that required a minimum core temperature of 56°C for a minimum of 30 minutes. This standard was based on the results of a multinational study involving Canadian, European, and American scientists (Dwinell, 1997). These time/temperature requirements were later used in ISPM #15 for the HT standard (FAO, 2006), however, this temperature/time requirement does not eliminate all exotic species. Morrell (1995) compiled a list of species that can survive the HT schedule found in ISPM #15. The vast majority of species found on the list are fungi that pose less of a risk than other organisms. Fungi are often restricted by environmental requirements, preventing them from becoming widespread pests (Hulme 1979). However, one thermotoleraent species of concern is *Phellinus weirri,* a root pathogen that can cause serious damage to forest ecosystems (Morrell, 1995).

Another drawback to heating is that it is virtually impossible to verify that the process has been carried out according to the IPPC standards, especially in material that is reused. In addition, Dwinell (1995) showed that heat-treated pine logs, placed in the field during the flight period of the pine sawyer beetle (*Monochamus sciuttolatus*) were re-infested with both the PWN and pine sawyers (Dwinell, 1995). Thus, HT of wood packaging materials does not provide permanent protection against possible invasive species. While these drawbacks are of concern to researchers, heating is still considered to be highly effective against most wood inhabiting insects and fungi. In addition, increasing the time/temperature relationship or requiring a substantial reduction in wood moisture content to make materials less attractive to insects would be economically prohibitive (Clarke, 2001; Araman *et al,* 2003; McLeod III *et al,* 1991).

Methyl bromide (MB) is one of the most widely used and studied fumigants for pest elimination. This chemical was discovered in the 1930s and is currently the only alternative accepted under ISPM#15 for the treatment of SWPM. MB has a low boiling point (3.6°C) and penetrates most materials quickly, yet has very low sorption. Fumigant sorption can affect commodity quality, making it important that chemical quickly dissipates from a material (Harris, 1963). These properties have led to the widespread use of MB for fumigating soil, commodities such as grains, and structures (Kramer, 1992).

MB is highly toxic to humans, and has no color or odor, making this chemical difficult to handle (Harris, 1963). As a result, chloropicrin, another fumigant is usually added at low levels as an awareness agent. MB is also less effective when used to treat coniferous species above the fiber saturation point (FSP), yet much of the SWPM is treated while wet (Cross, 1991). Like HT, MB does not provide long term protection against re-infestation, since the chemical is rapidly dissipated after treatment (Harris, 1963). Verification of MB treatment by the importing nation is also impossible, since there is little or no residual MB in the SWPM days or weeks after treatment (Ruetze and Liese, 1985). MB has also been listed as an ozone depleting substance under the Montreal Accord. The Montreal Accord states that developed countries will phase out MB use by January 2005 with the exception of pre-shipment and quarantine application, which are permitted until 2015 (CSIRO 2001).

Pressure treatment has been in use since 1838 when the Bethel or full-cell process was patented. Since that time, numerous oil and water - based chemicals have been employed to protect wood against deterioration using this process. Indeed, the pressure treatment process has a well known ability to protect sound timbers (Hunt and Garratt 1938). Pressure processes are used to treat nearly 360 million cubic meters of wood annually in North America, prolonging the service life of this material from a few years to decades (Morrell 2001; Nicholas 1973). The treating industry has a large capacity, allowing it to rapidly treat packaging material used in international trade at a relatively low cost. In addition, the presence of these chemicals would be verifiable by importing countries and this process would also be able to provide long term protection not provided by fumigation or HT. Some of the chemicals used in these processes are of concern, however, since a majority of SWPM does not enter landfills and is used for other purposes once its service life has come to an end. In the past, broadly toxic pesticides and fungicides have been used for conventional wood preservation. Increased concerns relating to human and environmental health effects have encouraged the use of less broadly toxic chemicals, such as boron, copper azole and alkaline copper compounds.

Pressure treatment has considerable potential for mitigating pest risks associated with SWPM, but there are a number of knowledge gaps associated with this method that need to be addressed before this process can be used. There is little knowledge concerning the emergence of established insects or fungi from treated material. Members of the Buprestidae are known to continue development in pressure-treated Douglas-fir poles and emerge through the treated shell. Furthermore, there is no evidence supporting the ability of pressure treatments to penetrate existing insect galleries, especially those that are tightly packed with frass. Pressure treatment tends to provide protection by placing high chemical retentions near the surface, producing a barrier that provides decades of performance. Long term heavy duty wood preservatives are unnecessary for SWPM since this material does not come into ground contact, is not exposed to highly decay prone environments and has a relatively short service life. As a result, it may be possible to use less toxic chemicals at lower chemical retentions to reduce both costs and the potential environmental impacts of treated SWPM (Morrell, 2004). Thus, the following study was conducted to determine if pressure treatment could penetrate frass-packed insect galleries and if this treatment prevented emergence of insects through the treated shell, when complete penetration was not achieved.

The following study was undertaken to address some of these questions as a first step in moving the pressure treatment process into ISPM 15.

2. Methods

Gallery penetration study: Western Redcedar (*Thuja plicata* D. Donn) heartwood infested with the beetle *Trachykele blondeli* Marseul was chosen for the gallery penetration study due to the refractory nature of the wood and the heavily packed galleries produced by the beetle larva. This beetle infests the heartwood of living western redcedar, making it relatively easy to collect infested material. Thirty samples (150mm wide by 50mm thick by 300mm long) were cut from green material and end sealed with a marine epoxy. This material was then treated with ACQ in a commercial treating facility to a target retention of 4.0 kg/m^3. Treating parameters were: 10 minutes vacuum at 50.8 kpa followed by 965 kpa of pressure for 1.75 hours utilizing a treating solution maintained at 29 °C. Thus, materials treated in this matter would not meet current ISPM #15 HT requirements. Following treatment, the samples were oven dried (103°C) and sliced lengthwise into 10mm thick strips (Figure 1).

The cut surfaces of the samples were sprayed with chrome azurol S according to AWPA Standard Method A3-00 for determining penetration of copper containing preservatives (AWPA 2004). The presence of copper was indicated by a dark blue color. Each sample was photographed using a five-mega pixel digital camera under natural light conditions, minimizing shadows. These images were used to assess preservative penetration in beetle galleries. The number of insect galleries visible on each 50 x 300mm face was counted along with the number of galleries that were completely penetrated by the treating chemical. These values were summed for the 15 sections from each piece and the mean percentage of galleries penetrated was calculated.

Barrier penetration study: Two mm thick Ponderosa pine (*Pinus ponderosa*) sapwood veneers were cut into 10mm wide by 100mm long strips. Groups of one hundred strips were vacuum treated with ACQ or disodium octaborate tetrahydrate (DOT) to target concentrations of 1.2 kg/m^3 or 0.4 kg/m^3, respectively. A third set of 100 veneer strips were pressure treated using an experimental treating system containing 30ppm imidacloprid, 300ppm 2-n-octyl-4-isothiazolone-3-one and 100ppm green dye (Sensient Colors Inc. Milwaukee Wisconsin).

Insects were then extracted from naturally infested Ponderosa pine boards over a 6 month period from late fall to early spring by carefully splitting the wood into small fragments using an axe and a splitting mall. Some of the 0.5m long samples were also cut into shorter segments when knots were present to ease the extraction process. The insect larvae became visible on the surface as the wood was split into smaller units, at which point they were collected by hand. In some cases, however, the insect larvae fell out of the exposed galleries after splitting.

The extracted larvae were placed on a synthetic diet described by McMorran (1965), but modified by eliminating the 10ml of water per 100ml of diet, doubling the amount of nutrient agar and adding 25 cm^3 of 30 mesh ponderosa pine wood flour per 100ml of media (Gardiner 1970). A 10mm thick layer of the media was then poured into 100mm diameter petri dishes. Once the media had hardened, a veneer strip was placed through the center of the perti dish and another veneer strip was placed 5-15mm way (depending on larvae size) forming a simulated treated envelope (STE). A depression approximately as long and wide as the larvae was cut into the surface of the media between the two strips of veneer. One larva was then placed in this opening and a layer of solidified media was placed on top of it. The larvae were then incubated in a dark chamber at 32°C. The larvae were observed on a 48 hour basis for 20 days. The time required for larvae to penetrate through the veneer and the time required until larval death occurred were of particular interest but other behavioral changes were also noted.

Initial trials assessed single veneers, but subsequent trials used several veneers to simulate thicker treatment barriers. Each treatment and barrier thickness was assessed on a minimum of 3 larvae. In addition, 4 larvae were exposed to the artificial diet modified with 0.5ml of chlorpyrifos, a known contact larvicide, per 100ml of media to determine if the composition of the diet affected the efficacy of the preservatives. These larvae were monitored on a 24 hour basis until death occurred.

3. Results and Discussion

Gallery penetration study: The treated shell on all of the samples was very shallow, confirming the refractory nature of the material. The penetration depth was much less than the 10mm penetration required under AWPA Standard U1-04 (AWPA 2004). However, the treating process completely penetrated 88% (95% confidence interval 81.5-94.4%) of the insect galleries found in 18 samples. There was no evidence that the copper component of ACQ diffused away from the treated galleries into the surrounding material (Figure 2). Thus, the presence of insect galleries greatly increase the overall preservative coverage through the depth of the sample but the increase was defined by the insect galleries. The lack of diffusion into the surrounding wood combined with the lack of a thick treated shell might allow insect larvae that have survived the initial treatment to complete their life cycle and emerge from the material by avoiding intermittent treated pockets.

Barrier penetration study: The larvae found during the extraction process varied greatly in size and developmental stage. However, more lower in-star larvae were found early in this study. A majority of the larvae removed later in the test had progressed into the later in-star stages, using larvae size as a guide. The activity level of the larvae also varied from fall to early spring. Larvae removed during the warmer periods in late fall and early to mid-spring were extremely active upon removal from the wood substrate, while little larval activity was observed immediately after removal during the colder winter months. However, activity increased greatly in winter collected larvae once they were exposed to temperatures in excess of 25°C for two to three hours, and, resembled that of the larvae removed during warmer periods. Ten larvae of various sizes were incubated in the dark at 32°C for four weeks without the presence of treated veneer to determine the short term effects of the diet on the larvae. These larvae exhibited no negative responses after this time, thus any abnormal behavior observed during the remainder of the study was attributed to the treating chemicals.

Five larvae each were then exposed to 2mm thick wafers containing ACQ, DOT, or imidacloprid or untreated control veneers. Healthy larvae required approximately eight days (four 48 hour periods) to penetrate the 2mm thick ponderosa pine veneers when the envelope was untreated or treated with ACQ or DOT (Figure 3) and none of the larvae died after contacting either the treated or the untreated veneers. The behavior displayed by the larvae in contact with an imidacloprid treated veneer differed substantially from that found with the other treatments. One larva required less then 48 hours to emerge from between the treated veneers. Later, the behavior of this larva and others that had penetrated the imidacloprid barrier became extremely aggressive. Two of these larvae excavated their way through the plastic petri dish and into the surrounding container, while one larva exited through the plastic petri dish and then tunneled into some soft plastic based material being used to support the petri plates. In contrast, another larva perished after 12 days without exiting through the veneer, while the remaining larva survived until the end of the study, but did not exit through the STE. The larvae did not differ significantly in size or appearance from the other larvae prior to the commencement of the study, making it difficult to explain the variable behavior.

Three larvae exposed to untreated veneers and three exposed to DOT treated veneers required less then 48 hours to tunnel through 4mm of veneer, while two larvae required less then 48 hours to penetrate a 4mm thick ACQ treated shell (figure 4). These data suggest that penetration time was not linearly correlated to barrier thickness. One larva exposed to the ACQ treatment perished 8 days after insertion without penetrating the veneer; however, cause of death could not be established due to excessive mold growth. Given the collection method, it is entirely possible that damage sustained during this process contributed to mortality.

Larvae required an additional 144 hours to penetrate the imidacloprid treated veneers. This was significantly longer than the time required to bore through the other treatments, including the 2mm veneers. The highly aggressive behavior describe earlier in association with larvae exposed to imidacloprid was repeated. As in the previous study, one larva survived the entire 20 day trial without exiting through the imidacloprid treated envelope. Once again, no cause could be established for this behavior.

The barrier trial was repeated once more using a 6mm thick barrier treated with either imidacloprid or ACQ (Figure 5). Limited larval availability resulted in only 4 and 3 replicates, respectively, for these tests. The thicker barrier did not significantly increase the time required for the larvae to penetrate through the wood. The average time required for penetration was 4 days for the ACQ treated material and 8 days for larvae exposed to the imidacloprid treated veneer. The aggressive behavior expressed by the larvae exposed to the imidacloprid treatment was also noted. Imidacloprid exposed larva, like the others, survived until the end of the 20-day period. This larva was then placed on a fresh petri plate that did not contain any biocide, where it later pupated. These results indicated that exposure to the treated barrier had little or no permanent effect on the beetles.

While these results indicate that the larvae were clearly capable of penetrating the treated barriers, there remained a question about whether the media had affected the chemical sensitivity of the larvae. For example elevated media pH might alter either biocidal activity or the ability of the organism to sorb the chemical. Larvae grown in media amended with 10,000 ppm of imidacloprid were unaffected, continued to feed heavily and bored through the plates, consuming, on average, 23.5 cm^3 of the diet. These larval also displayed the same aggressive behavior described earlier, with one of the larva exiting the petri dish by boring a hole through the lid. Media was also amended with 0.5ml of chlorpyrifos, per 100 ml of diet to determine if a contact insecticide would be effective in this environment. All of the larvae exposed under these conditions died within 48 hours. However, chlorpyrifos is probably not suitable for SWPM treatment due to its broadly toxic nature. The inability of the imidacloprid to kill the larvae was thought to have been associated with the pH of the media. Thus the pH of the diet was determined by testing the diet before the agar had a chance to solidify. This investigation found that the diet had a pH of 5.70, which was well within the tolerances for imidacloprid. Thus media pH did not reduce effectiveness.

The lack of apparent effects of the treated barrier on larvae activity was investigated further by examining the frass to determine if the insects had ingested the material. Frass (0.2g) produced by one larva from the 4 mm thick barrier treatment was examined under a dissecting microscope. The shape of the frass and the comingling of wood fibers with components of the artificial diet was of particular interest since comingled materials from the artificial diet and wood fiber with rounded edges would suggest that the wood had been digested by the insect, while frass with sharp edges and loose wood particles would suggest that the larva had merely removed the wood so they could pass through the area, perhaps in search of more palatable material. Likewise, the surface texture was of interest since it would also yield evidence suggesting digestion of the wood rather then the mere removal. Frass removed from petri dishes containing imidacloprid, ACQ, or DOT treated veneers contained wood particles with intact cell structure , sharp edges, and no evidence that wood particles were intermixed with the artificial diet. These results suggest that this material was not digested by the larva. The material appeared to have been mined and moved aside so that the insect could reach more desirable materials such as the artificial diet (Figure 6). In contrast, wood in frass produced by larvae exposed to untreated ponderosa pine had rounded edges suggesting digestion rather then the removal of the material and was commingled with remnants of the diet (Figure 7).

4. Conclusions

Wood preservatives applied using a full cell process penetrated a majority of the insect galleries, even in a refactatory species such as western redcedar, however, complete penetration could not be achieved. Thus an effective treatment barrier surrounding the untreated material is needed to prevent the egress of larva and adult insects. The three treatment chemicals investigated during this study failed to limit larvae mining, since they do not act as larvicides. Chlorpyrifos did control insects in SWPM however, this chemical has been withdrawn from the market because of its broad toxicity. Further research efforts are needed to identify larvicides that can be applied to wood products using pressure processes.

5. Literature

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Figures

Figure 1. Cutting diagram for the penetration samples showing the widest face of the treated cedar block and the location of the cuts to produce 10mm thick strips. The dissection of the samples in this manner allowed for the assessment of preservative penetration into the insect galleries through the thickness of the sample.

Figure 2. Example of ACQ penetration in western redcedar with *T. blondeli* galleries. The black color was caused by a reaction between Chrome Azurol S and the copper component of ACQ indicating that a majority of the galleries were penetrated by the preservative. Note that, the chemical did not diffuse away from the galleries.

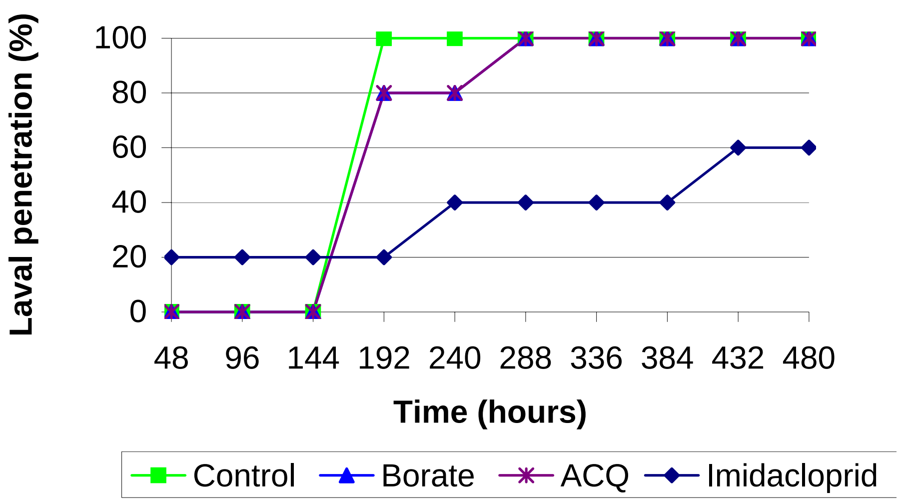

Figure 3. Percent of larvae boring through a 2mm thick untreated or preservative treated veneer at a given time after introduction. Observations were made on five larvae per treatment at the end of each 48 hour period.

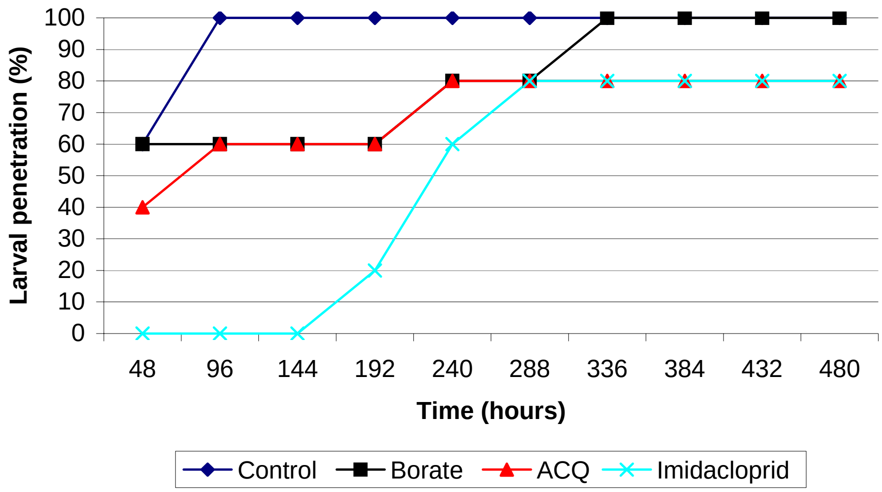

Figure 4. The amount of time required for insect larvae to penetrate 4mm thick simulated treated envelope.

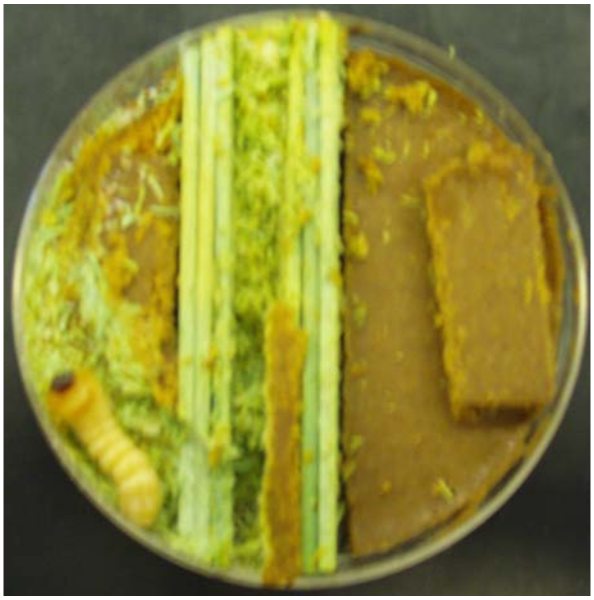

Figure 5. Petri dish containing larva that has penetrated a 6mm thick imidacloprid treated barrier 8 days after being introduced into this environment.

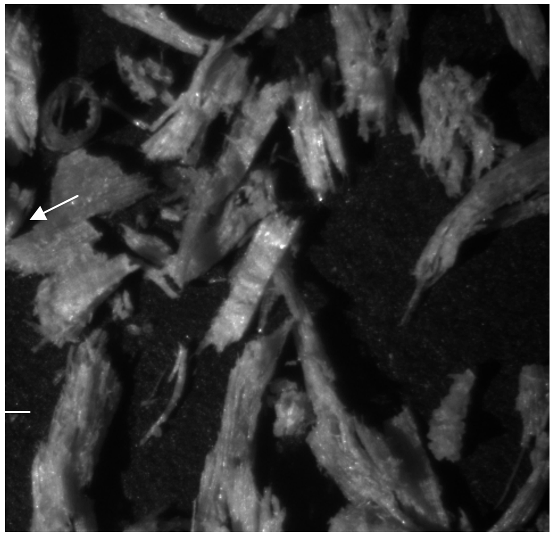

Figure 6. Micrograph of frass (10x) produced by a larva in contact with a 4mm thick imidacloprid treated veneer barrier showing wood with non-digested, sharp edged materials.

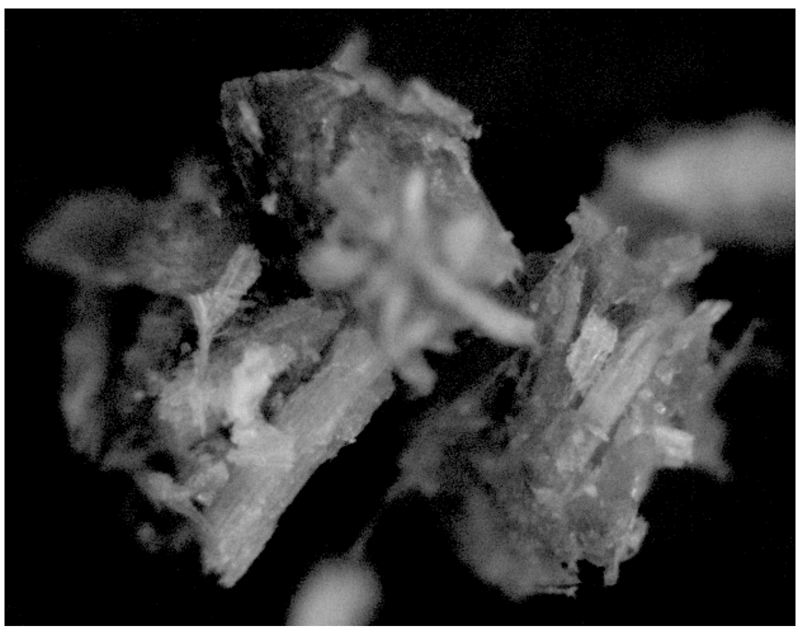

Figure 7. Micrograph (6x) of frass produced by a larva in contact with a 4mm thick ponderosa pine untreated veneer barrier with rounded edges on the individual particles suggestive of digestion of the wood.