Feasibility of eradicating cerambycid larvae and pinewood nematodes infesting lumber with commercial 2.45 GHz microwave equipment

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Abstract

This paper reports on work to investigate the feasibility to eradicate cerambycid larvae and the pinewood nematode (PWN) infesting lumber with commercial 2.45 GHz microwave equipment. Two specific goals within the experimental study were: 1) test a system of separating green material into moisture ranges and 2) determine the feasibility of killing the PWN pest and insect cerambycids applying commercial equipment using wood temperature as the critical treatment parameter. Prior research suggested moisture content would be a critical scale-up parameter (e.g. moving from laboratory-scale with small volume specimens) for full size lumber via any approved commercial treating process. Commercial units available to the study included: (1) a chamber design unit for batch irradiation treatment and (2) a continuous conveying tunnel design of microwave equipment fitted with optional air heaters. Microwave irradiation trials were conducted with red pine directly obtained from freshly sawn logs. Red pine was selected because of high wood moisture with strong influence on the subsequently required lethal microwave dosages. The completed trials conducted via the applied batch irradiation showed 100% morality in the all treated red pine materials (4- by 4- by 20-inch cant samples) infested PWN and cerambycid above a measured wood temperature above 63ºC, regardless of wood moisture. In contrast treatment of 1-thick boards (continuous feed microwave irradiation) it was found 100% morality was achieved at lower measured wood temperatures. However, this test observation needs further experimental verification. Overall, the results are encouraging to conclude that commercial microwave (2.45 GHz) treatment is a feasible alternative to conventional heat treatment or methyl bromide fumigation.

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Commercialization of microwave processes to eradicate exotic pest infestations in lumber may provide a useful tool for nations endeavoring to exclude these invasive pests from inadvertently crossing their borders in wood pallets and crates or other forms of solid wood packing material. Introduction of pinewood nematodes and cerambycids, such as the Asian longhorned beetle, *Anoplophora glabripennis* (Motsch.) (Coleoptera: Cerambycidae) (ALB), into new habitats threaten forest resources around the world (APHIS 1998, Nowak et al. 2001, Krehan 2002, Mota et al. 1999). Currently, heat treatment and methyl bromide fumigation are the only two sanitization treatments internationally approved for solid wood packing materials under the auspices of the United Nations (U.N.) (FAO 2000). However, the U.N. phytosanitary commission is also seeking alternative technologies, such as microwave energy, which can be used for this application. The goals of this study were to 1) test a system of separating nematode- or cerambycid-infested solid wood material (1- by 4- by 20-inch and 4- by 4- by 20-inch red pine) into moisture ranges by green weight and 2) determine the feasibility of killing nematodes and cerambycids in commercial equipment using temperature as the critical treatment parameter.

Microwave energy is used commercially for a number of applications such as cereal and granola baking, drying of coatings, snack food processing, foundry core drying, pharmaceutical processing, ceramic filter drying, ceramic sintering, rubber vulcanization, chemical vapor deposition, mold drying, and chemical waste processing (Cober 2004). In the wood products industry, TrusJoist (Strand 2002), a Weyerhaeuser company, consolidates veneer cut strands into processed composite lumber applying microwave energy. Mortality of termites, powder post beetles, and woodworm larvae in wood, as well as ALB larvae in living trees after microwave irradiation, has been studied (Burdette et al. 1975, Jiang et al. 1991, Andreuccetti et al. 1994, Lewis et al. 2000). Fleming et al. (2003, 2004) showed that microwave irradiation (2.45 GHz) is lethal to cerambycid larvae such as the ALB and the cottonwood borer beetle (CWB), *Plectrodera scalator* (Fabricius) (Coleoptera: Cerambycidae) in laboratory-size wood samples. Fleming et al. (2003) also showed that as the moisture content of the wood increased, additional microwave energy was required to ensure mortality of the larvae in wood.

Methods and materials

Equipment: Both continuous and batch, 2.45 GHz microwave systems provided by Cober Electronics, Inc. (Norwalk, CT) were used in this study. The continuous system illustrated in Figure 1 was designed for industrial applications. The microwave chamber was 10- by 10.5- by 72-inches long with an oven opening 8 inches wide by 2 inches high. A Teflon fiberglass conveyor belt (5 inches wide) was positioned approximately a 5-inch distance from the bottom of the chamber. Conveyor speed was variable for 0 to 100 feet/minute (fpm). Forced air, either room temperature or heated with an 18 kW electric resistance heater (maximum temperature 260ºC, 500ºF), flowed through the chamber at 1,000 fpm. Forced air temperature was measured with a thermocouple (type J). One 2 kW generator was connected to each of six portals (two located on the bottom of the chamber near the entry and exit openings and two staggered on each side of the chamber) for a combined maximum of 12 kW microwave power available. Due the request of the equipment owner, however, a maximum of 7 kW of input power was available for our experiments. A second limitation was that all experiments in the continuous system had to be conducted over the course of one day.

The batch system used in this study was designed for testing purposes, not for industrial use. The chamber dimensions were 28- by 28- by 20-inches high. A fan provided some airflow (non-measurable) through the chamber. One generator (6 kW, 2.45 GHz) supplied microwave power to the system (Figure 2a). A circular turntable approximately 20 inches in diameter was positioned 1 inch from the bottom of the cavity allowing load rotation if desired. Since the microwave field is at zero at the walls of the chamber and at the metal turntable, a 1-inch thick polymer disk also 20 inches in diameter was placed on top of the turntable so that the entire wood load would be irradiated. Without this polymer layer, the wood surface touching the metal turntable, as well as nearby bulk material, would experience a near-zero microwave field, as evidenced by minimal temperature increase measured in the wood (Fleming, unpublished data). Three fiber optic probes with a temperature monitoring range between –40 and 400 °C (Ipitech, Carlsbad, CA part no. 13-4600-0001) were threaded through a port in the

sidewall of the batch system chamber (Figure 2b). The phosphor tip and fiber optic cable are sheathed in a fluoropolymer with a total length of approximately 6 feet. Each probe was connected to a single channel, signal processing unit (LumiTherm® 500, Ipitech, Carlsbad CA). The LumiTherm® 500 system (Figure 3) uses a pulsed light source, which travels down the fiber optic cable and energizes the phosphor. Since temperature affects the time-decay of the phosphor glow, the sensor records the phosphor intensity and the signal processing system converts it into a temperature reading.

Insects: The CWB larvae used in this study were reared by the USDA Forest Service facility in E. Lansing, Michigan on a meridic insect diet (Payne et al. 1975, Fleming et al. 2003). Individual larvae were maintained in diet cups at room temperature until needed for experiments. At the time of irradiation, larval weight ranged from 0.80g to 2.25g. One larva was placed inside the pre-drilled hole in the wood sample and a wood plug was used to cap the hole (see Figure 4a). Once the sample was irradiated, the larva was removed and its status (living or dead) was determined by visual observation of: 1) movement or the lack of it, 2) discoloration, and/or 3) dehydration as described in Fleming et al. (2002). Figure 4b shows a CWB larvae with typical dehydration resulting from microwave treatment irradiation exposure.

Pinewood nematodes [*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle] were reared at The Pennsylvania State University, Fruit Research Center facilities in Biglerville, PA. Approximately 60 ml of white prose millet seed (Guerney's Seed and Nursery Company, Greendale, IN) and 75 ml of de-ionized, distilled water were placed in a wide-mouth, 16-ounce canning jar. The jar was covered with a glass petri dish and autoclaved for 15 minutes. After the jar was cooled, it was autoclaved again for an additional 15 minutes. Under sterile conditions, two agar plugs were transferred from a pure culture of fungus (non-sporulating *Botrytis cinerea*) to the cooled, canning jar with autoclaved millet seed. A glass petri dish was used as a lid and two-to-three wraps of parafilm were used to seal the top in order to reduce water loss. Inoculated jars were then kept in a high-humidity area for two to three weeks until the fungus covered the millet seeds. Once the fungus was established, five to ten agar plugs from a pure culture of pinewood nematodes were placed in the millet and the jars were resealed with parafilm. After approximately three weeks, one could usually observe the white fungus

disappearing and the color of the millet seeds darkening as the nematode population in the culture expanded. Nematodes could also be seen on the sides of the jar.

Pure cultures of nematodes were prepared in the following manner. Nine grams of bacto agar (Thomas Scientific, Swedesboro, NJ), 6-1/2g of potato dextrose agar (PDA) (Thomas Scientific, Swedesboro, NJ) and 500 ml of de-ionized, distilled water were placed with a stir bar in a 1-liter or larger flask that was covered loosely with an aluminum foil cap. Once the agar dissolved, the mixture in the flask was autoclaved for 20 minutes and then left to cool until the flask was warm to the touch. The mixture was then poured into petri dishes (approx. 20) under the sterilized hood. The dishes were covered with lids once all condensation dried. The pure cultures of fungus were prepared in the same manner, except there was no Bacto agar was added and a total of 19.5g of PDA was mixed with the 500 ml of de-ionized, distilled water.

At least 24 hours before the experiment, water from the tap (room temperature) was added to the nematode colony in the canning jar. The mixture of millet seed, nematodes, fungus, and tap water was then stirred gently with a plastic spoon, in order to suspend the nematodes in the water. A small piece of fine-mesh wire screen was then used to cover the top of the canning jar, in order to retain the millet while separating the nematode/water mixture into a holding container. The millet was rinsed again three or four times in a similar manner so that most of the nematode colony was captured in the holding container. The nematode/liquid was then poured into a No. 400 U.S.A. Standard Testing Sieve (ASTM E-11 specifications, 38 µm opening, www.advantechmfg.com) and rinsed under the tap, trapping the nematodes as the fungus and other small particles were washed away. In order to facilitate the separation of live from dead nematodes, a second sieve was constructed from PVC pipe fitted with an ultra fine mesh screen. This sieve was covered with a piece of kimwipe (1 ply, extra-low lint, Kimberly-Clark Corp., Roswell, GA) and placed inside a 6.5-inch diameter funnel with a clamped piece of 1-inch diameter tubing at the bottom (Fig. 5). The nematode mixture was then poured through the second sieve and additional tap water was added until the water line just touched the bottom of the second sieve. Live nematodes would then crawl through the paper and the mesh, while the dead ones were effectively left behind. Since pinewood nematodes cannot swim, they dropped to the bottom of the funnel. After four to five

hours of settling time, the funnel was tapped. If nematodes were left in the bottom of the funnel for too long, dissolved oxygen was depleted and the nematodes died.

Once the funnel was tapped and the water/nematode mixture was captured in a beaker, 25 µl of liquid were transferred onto a counting dish and additional tap water was added until the bottom of the dish was covered. Both the live and dead nematodes in the dish were observed and counted under a stereo, 20x magnification dissecting microscope (Nikon Model SMZ-2T) with reflected, oblique lighting. A multiple tally denominator counter (The Denominator Co, Inc., Woodbury, CT) was used for all experiments. The number of living nematodes inserted into a sample was estimated by taking the mean of the live nematodes counted in three separate samples. An alternate method used was to take the mean of three counts of each of two separate dishes.

The nematode solution (25 µl) was inserted into ¼ inch diameter (5/8 inch deep) that was drilled into the bottom of a plug (3/4 inch diameter by 2-3/4 inch long) (Fig 4). About 1/8th inch of the ¼ inch diameter was then placed in the hole, in order to keep the nematode solution inside. The entire ¾ inch plug was inserted into the larger solid wood block (4- by 4- by 20-inch) nematode-side down before irradiation. This configuration placed the nematodes in the center of the sample. After irradiation, the entire ¾-inch plug was removed from the wood blocks and shipped with the control plugs (no microwave treatment) overnight to Biglerville, PA for nematode recovery. All nematodes were recovered within 48 hours. In preliminary experiments, we found that nematode recovery did not differ before versus after shipment. After weighing the wood sample, the ¼-inch plug was removed from the ¾ inch plug.

To recover nematodes, the larger plug was split in half with a small knife so that the hole was split lengthwise. A squirt bottle filled with tap water was used to flush both sides of the hole. The water was captured in a beaker and the nematodes were allowed to settle for at least 10 minutes before the top liquid was poured off. The remaining nematode/water mixture was transferred into two counting dishes. The counting dishes were magnified with the stereo dissecting microscope and nematode status was recorded. Due to the number of samples irradiated per day, it was not possible to count all nematodes in every sample. Therefore, if a live nematode was observed in a given sample, the treatment was deemed ineffective and counting of that sample ceased. For a

small number of the samples, both the dead and live nematodes were counted. The plugs were then dried in a 40°C incubator for a minimum of 48 hours and weighed, in order to determine moisture content before treatment and after shipping.

Sample preparation: Freshly felled logs of red pine (*Pinus resinosa*) were bucked into 8-10 foot lengths for ease of handling and transport. In particular, red pine was specially selected for the study activities given its status as a primary host for the pinewood nematode. The harvested logs were subsequently sawn on a bandmill to recover solid wood materials to be used in the experiments. Sawn materials were further processed through jointing and planer equipment to prepare the final dimensioned wood samples and cut-to-length into 4- by 4- by 20-inch cant sized sections or dressed 1- by 4- by 20-inch boards. Processing efforts were taken to control the sample preparation to contain minimum amount of knots that could affect the desired placement of the wooden plugs that contained either the CWB larvae or nematodes. Holes in preparation for the insertion of either nematodes or cerambycid larvae were carefully drilled in the wood samples. For the 4- by 4- by 20-inch samples the holes were located 2 inches, 5 inches, 6 inches or 10 inches from the face (Fig. 6). Depending on the orientation of the samples in the chamber, the holes could be 2 inches from the front face (ff), 5 inches ff, 6 inches ff, 10 inches ff, 15 inches ff, 16 inches ff, or 18 inches ff. The holes for nematodes were drilled 2-$^{1}/_{8}$ inches deep and $^{25}/_{32}$ inch diameter, while the holes for larvae were drilled to the same depth, but were $^{17}/_{32}$ inches diameter. The fiber optic probes holes were drilled with a #49 diameter drill bit (0.073 inch) 1-$^{7}/_{8}$ inches deep within $^{1}/_{8}$ inch of the larva/nematode hole. For the 1- by 4- by 20-inch samples, one hole (2-$^{1}/_{8}$ inches deep, $^{17}/_{32}$ inches diameter) was drilled 10 inches from the face (Fig. 7). A second hole, 1/8th inch from and perpendicular to the larva/nematode hole, was drilled for the thermocouple probe (Omega, Model 881C digital multimeter).

Batch microwave system experimental design: Previous experiments on laboratory-size samples showed that wood mc affected the microwave dose required to obtain 100% mortality (Fleming et al. 2003). In order to minimize the microwave energy required to ensure that the nematodes/larvae were dead, loads should consist of wood with similar moisture content. Since moisture content (mc) calculations for each wood sample are based on oven-dry weight (ASTM 1996), actual wood mc could not be

determined for a green sample until after the experiment was completed and the sample was oven dried. In order to irradiate samples of similar mc together, the mc was estimated for each sample using equation [1].

$$SW_{green}=SG(mc+1)V_g \qquad [1]$$

where SW = weight of green sample (g)

SG = specific gravity (g/cm^3)

mc = fractional moisture content of sample

V_g = green volume of sample (cm^3)

The approximate specific gravity (SG) was estimated by averaging the SG calculated for 36, red pine samples (4- inch cubes) using ASTM standard D2395 Method A (ASTM 1996). A green SG value of 0.36 was determined for the cant material often obtained from the inner saw log portion. Since much of the study materials cut from log cores contained substantial proportions of juvenile wood, this value was used instead of the published SG for red pine, 0.41, which is for small, clear, straight-grained wood (USDA Handbook #72). SG estimation described above was used along with approximate volume for 4- by 4- by 20-inch wood sample material to construct a spreadsheet for expected green weights corresponding to 5% mc intervals. Moisture level ranges were then designated into groupings as follows: very high (VH), high (H), medium (M), and low (L) (Table 1). After initial processing into sample material, each sample piece was weighed (nearest gram weight basis) and grouped into one of the targeted mc ranges.

Three configurations of the 4- by 4- by 20-inch samples containing larvae were tested to determine which was the optimum configuration for the chamber (see Fig. 8). Other samples did not have larvae inserted in them. In configuration a, the boards were stacked 3 high, 4 across with wood spacers (approximately 1- by 1- by 20-inch.) between the rows. Fiber optic probes were placed in the wood sample 2 inches deep and within 1/8 inch from the larvae in A1, B3, and C4. In configuration b, boards were stacked 2 high and 4 across also with wood spacers between the rows. Fiber optic probes were placed in the sample near A2, B1 and B4. In configuration c, boards were stacked 1 high and 4 across. Fiber optic probes were placed in the sample near A1, A2, and A4. With

this chamber, the load could not be turned on the turntable or the fiber optic probes would be broken. Input power of 1000 W (1kW) was used to irradiate configurations a and b, while 3000 W was the input power applied to configuration c. Due to temperature variations in the wood (Fig. 9), configuration c was chosen for the rest of the experiments (N=45) in the batch microwave system.

Four to six loads for each mc range (VH, H, M, and L) of red pine infested with larvae were irradiated at 5000 W until all fiber optic probes registered at least 62 °C. After this critical temperature was reached, the plug was removed from each sample. Each larva was then extricated and its status (live/dead) was determined. For nematode experiments, the samples (5 loads of VH, M, and L mc ranges, 10 loads of H mc) were also irradiated at 5000 W until probes registered at least 62 °C. Once removed from the sample, the nematode plugs were shipped to Biglerville, PA overnight in an insulated freezer bag with ice packs. Recovery and counting of live nematodes was completed within 72 hours of microwave treatment.

Continuous microwave system experimental design: Red pine samples (1- by 4- by 20-in. long) were separated by green weight into three groups with average weights of 979g (867g-1103g), 1201g (1115g-1319g), and 1402g (1207g-1680g), referred to as medium, high and very high green weight, respectively. One run (n=10) from each group contained nematodes, while the other runs contained cerambycid larvae. Boards were placed on the moving conveyor belt (end-to-end in order to have a consistent load inside the chamber at any one moment in time. The high (n=30) and very high (n=20) weight samples were irradiated at 7 kW, while the medium weight samples (n=60) were irradiated at 6 kW and 5 kW. Because the belt was moving at 2.6 ft/min, total time in the 6 ft chamber for a single sample was 2.31 min. Due to system design, forced air was always circulating through the chamber at 1000 fpm while the equipment was in operation. We observed in test runs with non-infested wood of high and very high green weight that the wood temperature was not consistently reaching 63ºC at the maximum input power of 7 kW, even at the slowest conveyor belt speed. With chamber heat added, however, interior wood temperature in the test samples was generally higher. Therefore, we ran some runs with chamber air temperature at 33ºC (91ºF) and others at 110ºC (230ºF). After the sample came off the belt, a thermocouple was immediately placed

inside the predrilled hole and a fiber optic probe was placed on the surface in order to register the internal and surface temperatures. The larvae or nematodes were then removed from the sample and their status (living/dead) was determined in the same manner as the batch experiments described above.

Results and discussion

Batch microwave experiments: The average, maximum, and minimum mc for each group of wood (VH, H, M, and L) were calculated and reported in Table 2. Comparison of the actual sample mc to the predicted wood mc using green weight and either the SG value of 0.36 obtained through sampling or the published SG value of 0.41 in Equation [1] did not produce accurate results (paired t-test: $t = -14.0$, $df = 124$, $p < 0.0001$; $t = 2.24$, $df = 169$, $p = 0.0262$, respectively). There was, however, a strong, linear relationship between moisture content as predicted by Equation [1] and actual moisture content for both SG values of 0.41 and 0.36 (Linear Regression: $F = 986$; $df = 1,68$; $p < 0.0001$, $R^2=0.85$; $F = 768$; $df = 1, 123$; $p < 0.0001$; $R^2 = 0.86$, respectively). Thus, by adjusting the predicted mc values obtained with Equation [1] with the following linear equations, mc can be predicted with an accuracy of 85-86% (Fig. 10):

SG = 0.41, adjusted predicted mc = 14.4 + 0.78*(predicted mc); [2]

SG = 0.36, adjusted predicted mc = 7.2 + 1.24*(predicted mc) [3]

Regardless of mc, there was 100% mortality of cerambycid larvae in red pine samples that reached 62°C. At this same temperature, however, some nematodes in red pine samples survived (Table 3). When wood temperatures registered above 63°C, 100% nematode mortality resulted. Recovery rate for nematodes in the control plugs ranged from 9% to 66%. All control samples contained live nematodes (Table 4). A similar recovery rate of 6 to 31% was achieved for the treated samples. Within the subset of samples where all recovered nematodes (both live and dead) were counted (Table 5), there was no correlation between percent mortality and percent recovery ($p = 0.729$), nor was there a relationship between percent recovery and live nematodes ($p = 0.222$), indicating that there was no bias in the recovery of live vs. dead nematodes. There was a

significant linear relationship between temperature and mortality (Linear regression: $F = 14.5$, $df = 1,46$, $p = 0.0004$; equation: arcsin(mortality) = 0.91 + 0.0077*(temp); $R^2=0.24$). Mortality was significantly higher in wood samples that reached 62°C or above compared to untreated controls (ANOVA followed by orthogonal contrasts, $p = 0.0002$)). Also, the mc of treated wood did not affect the percent recovery of nematodes ($R^2 = 0.11$, $F = 3.5$, $df = 1, 28$; $p = 0.0721$), suggesting that this relationship should hold for high, medium, or low MCs. This is likely due to the fact that the nematodes were only left in the wood (treated or untreated) for a maximum of 48 hours.

As moisture content increased, the time required for wood samples to reach 62°C increased (Linear regression: $F = 158$; $df = 2, 146$; $p < 0.001$; $R^2_{adj} = 0.68$). In addition, position of the temperature probes along the wood sample had a minor effect on time required to reach 62°C. Positions 2 and 4 in. away from the end of the board reached 62°C more slowly than positions 6 and 8 in. away from the end of the board (Equations: $\log(time)_{sec} = 2.42 + 0.605*(MC)$; $\log(time)_{sec} = 1.825 + 0.605*(MC)$, respectively). The slope of the time required to reach 62°C was the same regardless of position of the probe, but the intercepts were significantly different. Since the samples were stationary in the batch mw system, the mw field intensity varied within the chamber and distribution of mc within each board likely varied, contributing to the slight differences in time to reach this temperature along the length of the boards.

Continuous microwave experiments: Not all wood samples reached the target internal temperature of 62°C in any of the mc groups (Table 6) and yet mortality using continuous MW energy killed all nematode and larval samples. In addition, when data for all wood moisture contents were combined for analysis, mc prior to treatment with continuous MW energy was negatively correlated with internal wood temperature (Linear Regression: $F = 16.5$; $df = 1,68$; $p = 0.0001$) (Table 6). When wood was sorted by mc for analysis (medium, high and very high), however, green weight was not predictive of internal wood temperature, especially for wood of very high mc ($F = 0.47$; $df = 1,18$; $p = 0.502$; mean internal temperature = 58.5 ± 1.2 (SEM), which in part may be due to smaller sample sizes when groups were analyzed separately. For the medium and high mc wood groupings, although there was a negatively correlated trend, mc was not significantly predictive of internal temperature (medium mc: $F = 1.82$; $df = 1, 18$; $p =$

0.194; high mc: $F = 1.79$; df = 1,28; $p = 0.194$). Despite this finding, the mean internal temperature of medium and high mc samples exceeded the target of 62°C (69.8 ± 2.50 and 65.2 ± 1.98°C, respectively). One possible explanation for the low temperatures recorded for some samples may be that the total mc load in the chamber is influenced by the total mc load from all of the boards combined, not just by the mc of each individual board. For future experiments we intend to investigate this hypothesis.

Live nematodes were recovered from all control plugs, whereas 100% mortality was obtained for all larvae and nematodes in treated samples of 1- by 4- by 20-in. dimensions. Core wood temperatures as low as 46°C and 53°C were still lethal to larvae and nematodes, respectively. These lower temperature requirements may be due to the movement of the sample through the chamber, thus exposing the sample to a more uniform mw field. Additional trials conducted on 4- by 4- by 4-inch wood cubes tended to verify almost a 100% mortality of cerambycid larvae was possible at lower temperatures when the samples are rotated compared to samples that remain stationary during mw treatment. These trials were conducted in a separate noncommercial scale 2.45 GHz microwave unit. Nematode mortality in counted samples treated with 7 kW of power in a heated 110°C or 33°C chamber were significantly greater than untreated controls ($\chi^2 = 26.5$, df = 1, $p < 0.001$ and $\chi^2 = 15.2$, df = 1, $p = 0.001$). However, the wood used in these runs had different average moisture contents, so direct comparisons between heated and unheated chambers have yet to be tested.

Conclusions

In the batch experiments where the mc load and the mw input power were fixed, increased wood mc increased the mw exposure time required to reach 62°C. Thus, separation of lumber by mc will likely be an important aspect of the treatment process in order to reduce the mw energy input required for 100% mortality in a batch. Untreated red pine lumber can be grouped by estimated mc using Equations [1] and [2] or [3], as long as the green weight and the volume of the board are known. We expect that similar equations can be developed for other wood species. Additional experiments are planned to test this separation method further.

Wood temperatures greater than 62°C are lethal to pinewood nematodes and cerambycid larvae infesting red pine in a chamber in which the wood samples remain stationary. Our preliminary findings also suggest, however, that nematodes or larvae in lumber in which the mw field is continuously moving, either by rotation or on a conveyor belt, die at lower wood temperatures than in a batch system without movement. We recommend that additional experiments with non-stationary, commercial equipment be conducted to investigate this phenomenon further. Additional assessment of the effects of conventionally heated vs non-heated mw chambers on lethal mw doses would also be helpful for the commercialization and regulatory process.

We can conclude from our experiments that commercial mw treatment (2.45 GHz) of 1-inch thick red pine lumber infested with cerambycids or pinewood nematodes is a feasible alternative to conventional heat treatment or methyl bromide fumigation.

Literature cited

American Society for Testing and Materials (ASTM). 1996. Standard test methods for specific gravity of wood and wood-based materials. ASTM Standard D 2395. Annual Book of ASTM Standards, Vol. 4.10. ASTM, West Conshohocken, PA.

Andreuccetti, D., M. Bini, A. Ignesti, A. Gambetta, and R. Olmi. 1994. Microwave destruction of woodworms. International Microwave Power Institute. 29(3):153-160.

Burdette, E.C., N.C. Hightower, C.P. Burns, and F.L. Cain. 1975. Microwave energy for wood products insect control. Microwave Power Symposium Proceedings, Manassas, VA. 10:276-281.

Cober Electronics Inc. 2004. Brochure. 151 Woodward Ave., Norwalk, CT http.//connix.com/~myone/cober/cober.htm.

Fleming M.R., K. Hoover, J.J. Janowiak, Y. Fang, X. Wang, W. Liu, Y. Wang, X. Hang, D. Agrawal, V.C. Mastro, D.R. Lance, J.E. Shield, and R. Roy. 2003. Microwave

irradiation of wood packing material to destroy the Asian longhorned beetle. Forest Prod. J. 53(1): 46-52.

Fleming M.R., J.J. Janowiak, J. Kearns, J.E. Shield, R. Roy, D.K. Agrawal, L.S. Bauer, D.L. Miller, and K. Hoover. 2004. Parameters for scale-up of lethal microwave treatment to eradicate cerambycid larvae infesting solid wood packing material. Forest Prod. J. 54(7/8): 80-84.

Jiang, S.D., G.X. Wang, Z.Z. Zhang, and Y.Z. Li. 1991. A preliminary study on the control of some stem borers of trees using microwave technology. Forest Pest and Disease. 1:20-22.

Krehan, H. 2002. Asian longhorned beetle in Austria: critical comments on phytosanitary measures and regulations. *In Proc.* U.S. Department of Agriculture Interagency Research Forum On Gypsy Moth and Other Invasive Species, General Technical Report NE-300. S.L.C. Fosbroke and K.W. Gottschalk (eds.), USDA, Forest Service, Northeastern Research Station. Newtown Square, PA. pp. 5-6.

Lewis, V.R., A.B. Power, and M. Haverty. 2000. Laboratory evaluation of microwaves for control of the western drywood termite. Forest Prod. J. 50(5): 79-88.

Mota, M.M., H. Braasch, M.A. Bravo,A. C. Penas, W. Burgermeister, K. Metge and E. Sousa. 1999. First Report of *Bursaphelenchus xylophilus* in Portugal and in Europe. Nematology 1(7-8):727-734.

Nowak, D.J., J.E. Pasek, R.A. Sequeira, D.E. Crane, and V.C. Mastro. 2001. Potential effect of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on urban trees in the United States. J. Econ. Entomol. 94:116-122.

Payne, J.A., H. Lowman, R. R. Pate. 1975. Artificial diets for rearing the tilehorned *Prionus.* Ann. Entomol. Soc. Am. 68:680-682.

Strand, R. TrusJoist. Boise ID. www.trusjoist.com, personal communication 7/19/02

United Nations (FAO). 2002**.** Guidelines for regulating wood packaging material in international trade, pp. 1-12. International Standards for Phytosanitary Measures, March 2002. Publication No. 15. Food and Agriculture Organization of the United Nations, Rome, Italy.

United States Department of Agriculture Animal, Plant Protection and Quarantine (APHIS). 1998. Solid wood packing material from China; interim rule 7CFR319-354. http://www.aphis.usda.gov/oa/alb/interimalb.html; 1-58.

Table 1: Targeted mc and predicted green weight (0.38 and 0.41 specific gravity) of the red pine samples with 4- by 4- by 20-in. dimensions for each of the four mc ranges.

Sorted Grouping 4- by 4- by 20-in. Samples	**Targeted mc (%)**	**Predicted Green Weight (g)** (0.36 specific gravity)	**Predicted Green Weight (g)** (0.41 specific gravity)
Very High	VH > 150	VH> 4720	VH > 5375
High	110 < H < 145	3964 < H < 4625	4515 < H < 5267
Medium	65 < M < 105	3114 < M < 3870	3547 < M < 4407
Low	L < 60	L < 3020	L < 3440

Table 2: Average, maximum and minimum mc, as well as standard deviation, calculated for each mc grouping (VH, H, M, and L assuming 0.36 specific gravity, SG) of 4- by 4- by 20-in. samples.

Sorted Grouping	**Mean actual mc (%)**	**Maximum actual mc (%)**	**Minimum actual mc (%)**	**Standard deviation**	**Coefficient of variation (%)**
4- by 4- by 20-in.					
VH	120	178	86	23	19.1
H	84	118	71	9	10.7
M	67	84	43	11	16.4

L 36 47 25 6 16.6

Table 3: Summary of nematode experiments treated in the batch mw system.

Wood temperature (°C)	Total Number of Wood Samples	Number of samples containing live nematodes
25 (controls)	18	18
< 62	5	2
62	29	2
> 62	66	0

Table 4: Number of live nematodes recovered from control plugs daily.

Day	No. of control plugs per day	Avg no. of nematodes inserted per plug	No. of live nematodes recovered from plugs (min/max)
1	6	160	4/55
2	8	351	4/217
3	4	272	7/9

Table 5: Mean nematode mortality by wood temperature (batch mw system) for samples whereboth live and dead nematodes were counted (n=48). Means followed by the same letter are not significantly different at the $p < 0.05$ level.

Wood temperature (°C)	Mean nematode mortality (%)	Standard deviation
25 (controls)	77^{a}	28.7
< 62	33.3^{a}	26.7
62	26.7^{b}	57.7
> 62	100^{c}	0

Table 6: Wood temperatures and mortality of larvae (L) and nematodes (N) by continuous microwave treatment of 1-by 4- by 20-in.
boards.

n	L or N	Mean ± SEM green wt. of sample(g)	MW power (kW)	Chamber air T (ºC)	No. of boards	Internal wood T(ºC) ± SEM	Internal wood T range (ºC)	Surface wood T (ºC) ± SEM	Surface wood T range (ºC)	No. of L or N alive a treatme
trol	N		0	25	4		NA		NA	2-12
	N	975 ± 22.0[a]	6	110	10	84 ± 3.0	74-95	50 ± 2.5	44-61	0
	N	1222 ± 16.8[b]	7	33	10	60 ± 1.6	53-69	44 ± 1.3	40-50	0
	N	1455 ± 43.0[c]	7	110	10	60 ± 1.6	54-66	44 ± 0.6	41-47	0
	L	983 ± 21.8[a]	5	110	10	76 ± 2.2	70-91	48 ± 1.6	42-58	0
	L	978 ± 15.4[a]	6	33	20	70 ± 2.5	53-95	NA	NA	0
	L	996 ± 21.8[a]	6	110	10	81 ± 2.2	69-94	51 ± 1.3	46-58	0
	L	1181 ± 16.5[b]	6	110	10	72 ± 3.5	58-88	51 ± 2.2	45-66	0
	L	1202 ± 19.6[b]	7	33	10	63 ± 3.8	46-86	43 ± 1.0	38-47	0
	L	1349 ± 33.2[c]	7	110	10	58 ± 1.9	51-72	43 ± 0.6	40-46	0

[a]medium green weight, [b] high green weight, and [c]very high green weight groups

Fig. 1a – Photograph of continuous 2.45 GHz industrial microwave used for experimental trials (outfeed side with core temperature measure being taken on 1- by 4-inch board samples).

Fig. 1b – Continuous 2.45 GHz microwave unit top
opened to show tunnel with belt conveyor.

29 8:41 AM

Fig. 2a – Batch microwave unit (2.45 GHz generator-left and treating chamber-right) used in experiments with 4- by 4-inch cant section samples.

Fig. 2b – Inside chamber view with installed fiber optic probes to monitor temperature elevation of irradiated red pine samples.

20 9:09 AM

Fig. 3. Fiber optic probe cables attached to signal processing units used to monitor temperature elevations inside the microwave chamber during sample irradiation.

Fig. 4a – CWB lavae being inserted into drilled hole with wooden plug installed immediately prior to the microwave irradiation treatment.

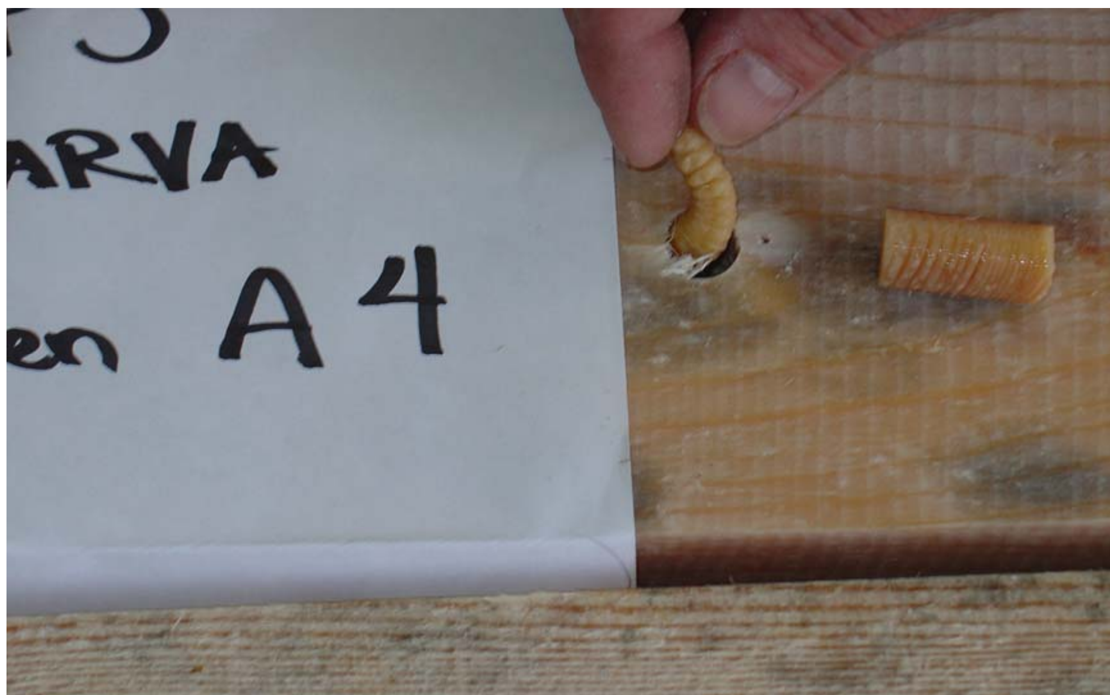


Fig. 4b – Dehydrated CWB larvae after microwave irradiation often observed in the treated red pine wood samples.

Fig. 5 – Positions of the holes drilled for nematode or larva insertion in the 4- by 4- by 20-inch red pine board a) 2 inches from the face and b) 10 inches from the face. Other hole positions not depicted included 5 inches and 6 inches from the face. Fiberoptic probe holes were also drilled within 1/8 inch of the larva/nematode hole.

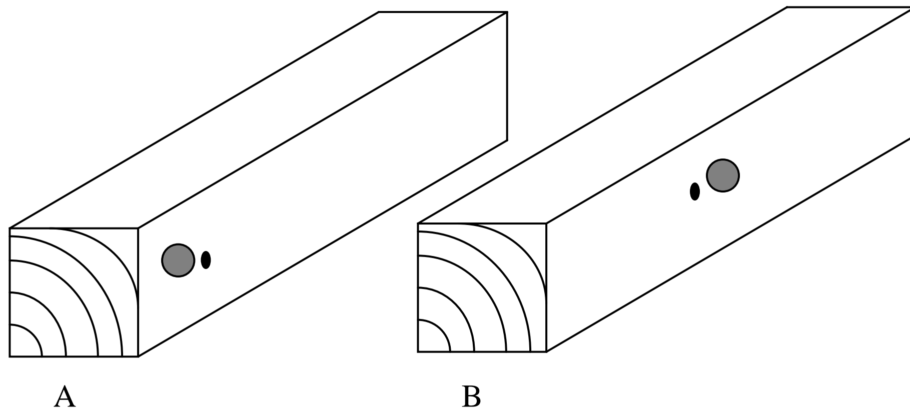


Fig. 6 – Positions of the holes drilled for nematode or larval insertion and the thermocouple probe in the 1- by 4- by 20-inch red pine board.

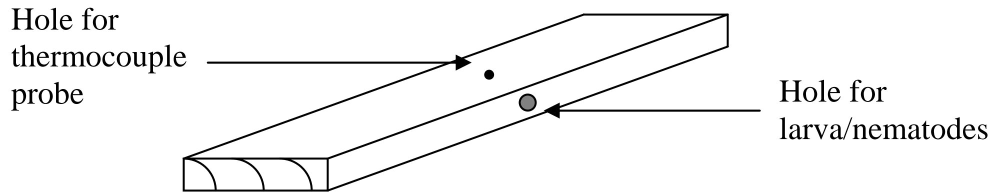


Fig. 7 — Diagram of the funnel set-up for separating live from dead nematodes.

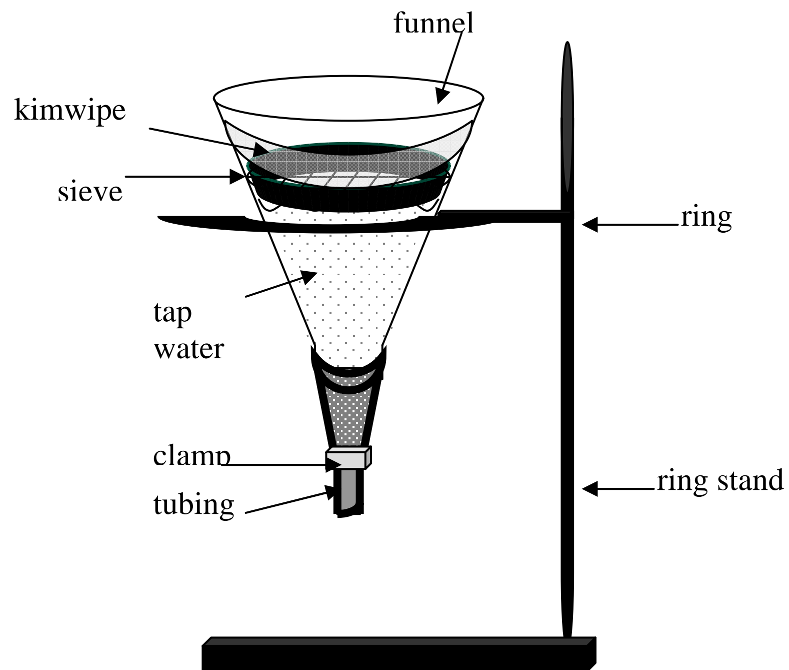


Fig. 8 – A ¼ inch hole was drilled in the center of a ¾ inch diameter, red pine plug. The nematode solution (25 mL) is inserted into the hole and then the hole is plugged with a ¼ inch plug. The entire ¾ inch plug is inserted into the larger, red pine sample (4- by 4- by 20-inch) nematode-side down before irradiation.

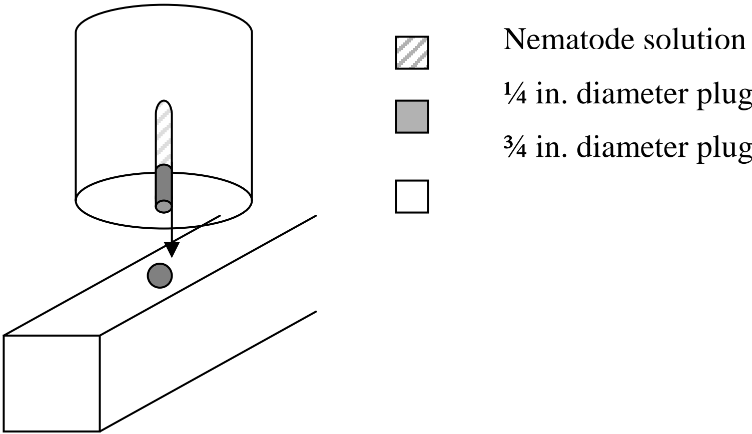
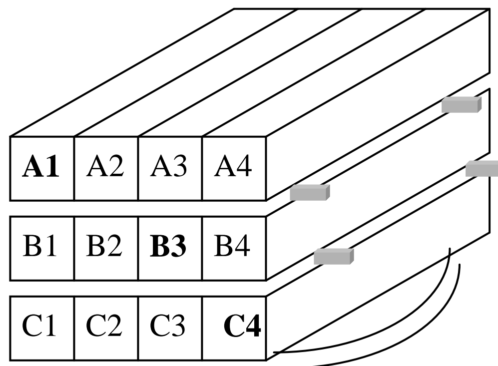
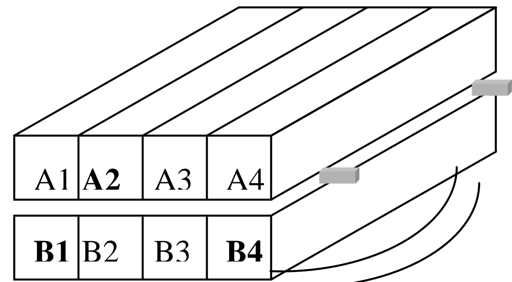


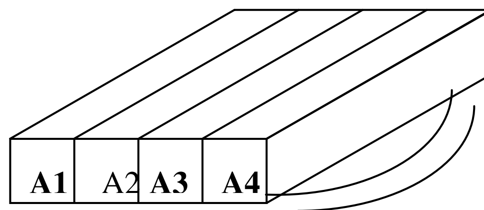
Fig. 9 – The three configurations of 4- by 4- by 20-inch boards with larvae tested for the optimum configuration in the chamber. a) 3 boards high, 4 boards across with wood spacers between the rows. Larvae were inserted in A1 (2 in. from front face, ff), B2 (6 in. ff), B3 (10 in. ff), C4 (15 in. ff), A3 (10 in. ff), and C3 (2 in. ff). All other boards did not have larvae inserted in them. Fiberoptic probes were placed in the wood sample 2 in. deep and within 1/8 in. from the larvae in A1, B3, and C4 (marked in bold), b) 2 boards high, 4 boards across with wood spacers between the rows. Larvae were inserted in A1 (2 in. ff), A2 (10 in. ff), B3 (6 in. ff), and B4 (15 in. ff). Nematodes were inserted in A3 (10 in. ff), A4 (18 in. ff), B1 (5 in. ff) and B2 (16 in. ff). Fiberoptic probes were placed in the sample near A2, B1 and B4. and c) 1 board high, 4 boards across. Either larvae or nematodes were placed in A1 (5 in. ff), A2 (16 in. ff), A3 (10 in. ff) and A4 (18 in. ff). Fiberoptic probes were placed in the sample near A1, A2, and A4.



Configuration
a



Configuration
b



Configuration
c

Fig. 10 – Batch mw experiment graphs (irradiation time vs temperature) for each temperature monitoring position and configuration as depicted in Fig. 5: A) configuration a (1000 W, 4- by 4- by 20-inch), B) configuration b (1000 W, 4- by 4- by 20-inch), and C) configuration c (3000W, 4- by 4- by 20-inch).

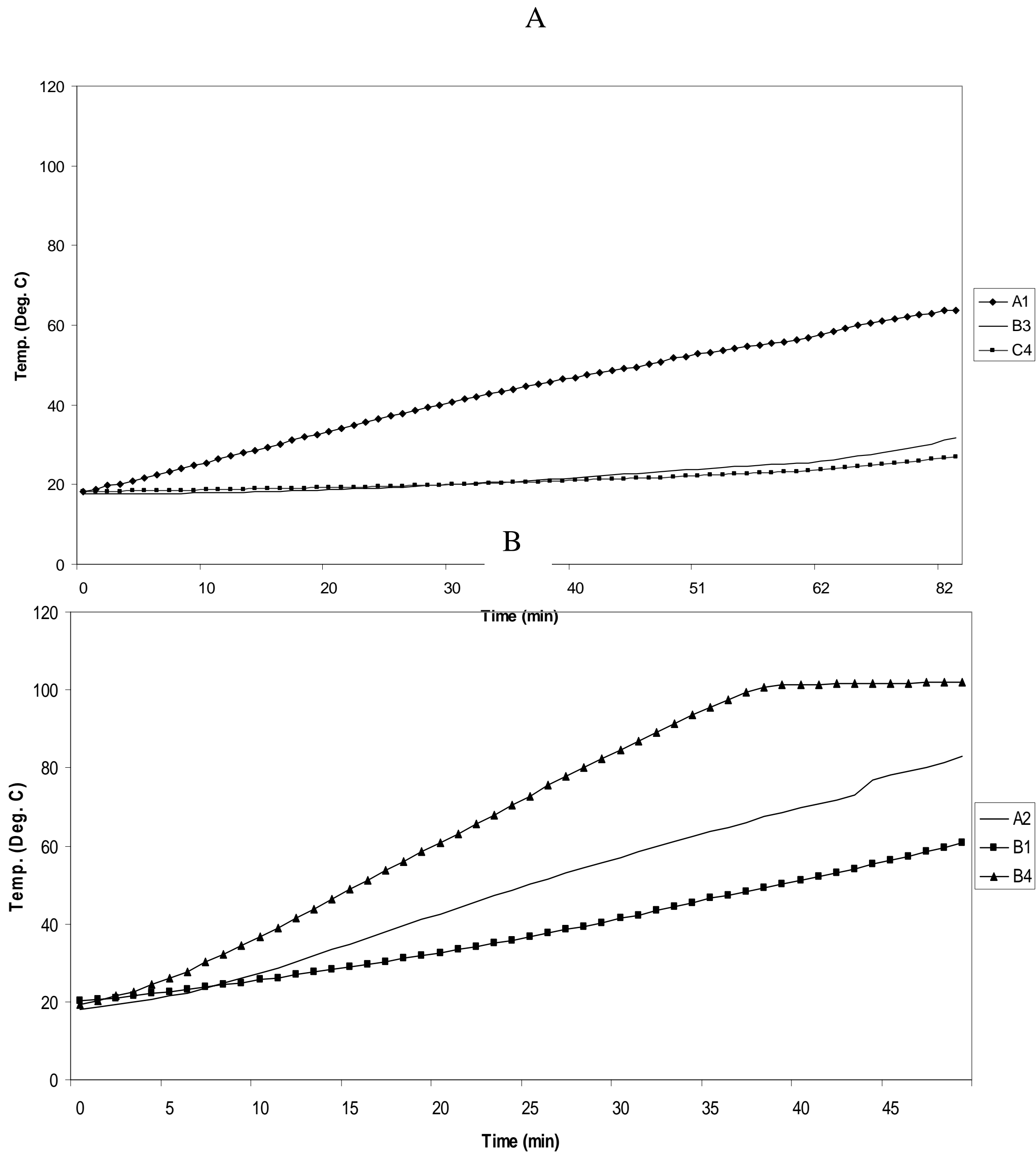


Fig. 10 -- cont.

C

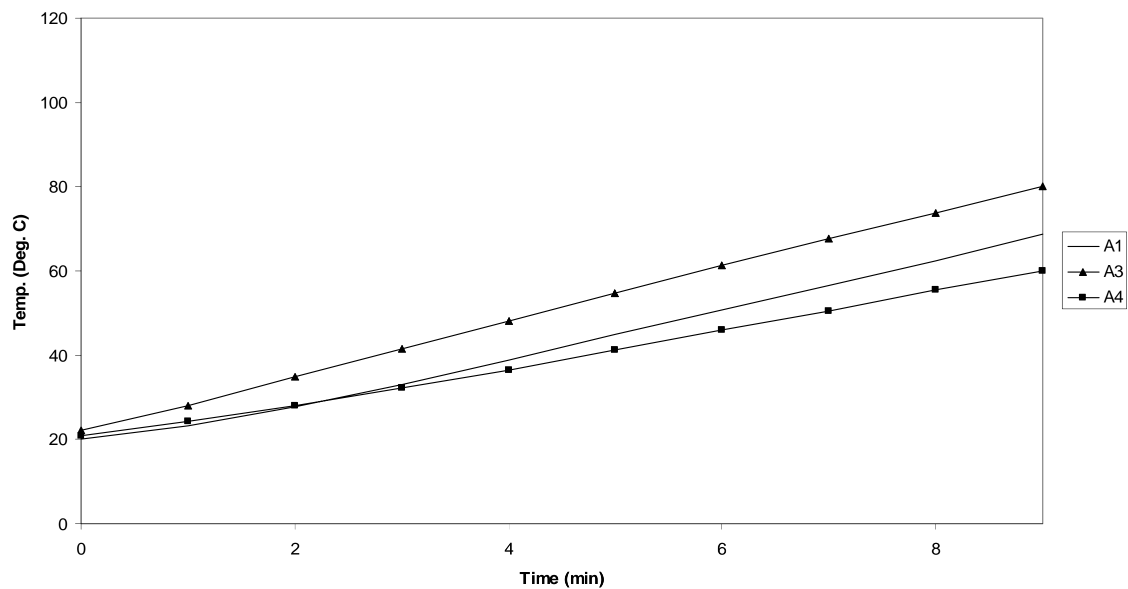


Fig. 11 – Relationship between predicted wood mc and actual mc for SG = 36 and 41.