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Efficacy of radio frequency treatment and its potential for control of sapstain and wood decay fungi on red oak, poplar, and southern yellow pine wood species

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Abstract The effectiveness of radio frequency (RF) treatment in the control of wood decay fungi (*Gloeophyllum trabeum*, *Ganoderma lucidum*, and *Irpex lacteus*) and sapstain fungus (*Ceratocystis fimbriata*) in red oak (*Quercus* spp.), poplar (*Populus alba*), and southern yellow pine (*Pinus* spp.) was evaluated in the laboratory as an alternative to methyl bromide (MB) treatment. Wood samples ($15.5 \times 10 \times 10$ cm) were inoculated with fungi from a 7-day culture by dipping them to a depth of one face deep (2 cm) into inoculum and incubating them at 25°C for 14 days. Identical wood samples were left uninoculated as controls. Subsequent to incubation, the wood blocks were exposed to RF radiation in an industrial 40-kW dielectric oven at temperatures between 60° and 70°C for 2 min. The test fungi were recovered and reisolated from all of the control wood blocks but not from RF-treated wood blocks. RF treatment resulted in complete inhibition of the fungus in 98%–100% of the wood samples. Moisture content loss (≥1%) was noted after wood had been exposed to RF treatment. Moisture content may be an important factor to consider with RF treatments. RF treatment can, therefore, potentially provide an effective and rapid quarantine treatment as an alternative to MB fumigation for certain pathogen–wood combinations.

Key words Radio frequency · Decay fungi · Sapstain fungi

Introduction

Wood decay is of tremendous economic significance to the forest industry as well as other sectors of the economy in the USA. Biodegradation of wood is accomplished in part by insects and marine borers, but the greatest degree of deterioration and product devaluation is caused by wood-inhabiting fungi.[1,2] Solid wood packing material (SWPM) is recognized as a major pathway for introduction of insects and pathogens into the USA on indigenous wood species used as packing material.

Currently, exported SWPM are disinfested using methyl bromide (MB) fumigation[3] or conventional heat sterilization treatment.[4,5] Restrictions on MB use[6] have increased interest in developing alternative treatments for SWPM. Radio frequency (RF) treatment has been used successfully in food processing, textile industries, and in many entomological and plant pathological studies for the control of insects[7–10] and plant pathogens.[11–14] In RF treatment, the electromagnetic energy interacts directly with the commodity's interior to quickly raise the center temperature.[15–17] Radio frequency treatment in an oven may be an alternative that can reduce adverse thermal impact of treated commodities during conventional heating processes.[18–20]

Previous studies have shown that radio frequency/vacuum (RF/V) drying technology can be used successfully for treatment of hard-to-dry species and thick timbers that cannot be dried using conventional methods;[11–14] however, its application in the field of plant pathology is very limited. Few studies have been conducted on the eradication of wood decay fungi using RF/V treatment.[18,19,21–23] Fang et al.[24] eradicated *Gloeophyllum trabeum* (Persoon: Fries) and *Postia placenta* (Fries) Cooke after 2 h of heating above 65°C.

The application of RF technology by Cwiklinski and Von Horsten[25] led to a complete eradication of *Fusarium culmo-*

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Table 1. Wood species, wood size, and initial moisture content of wood exposed to radio frequency (RF) radiation in an industrial 40-kW dielectric oven

Wood species	Block size (cm)	Treatment	Total wood blocks	Initial moisture content (%)
Red oak	15.5 × 10 × 10	Control	10	>100
		RF treated	30	>100
Poplar	15.5 × 10 × 10	Control	10	>100
		RF treated	30	>100
Southern yellow pine	15.5 × 10 × 10	Control	10	16.1
		RF-treated	30	16.1

rum (Smith) Saccardo in seed while maintaining germination. They reported complete eradication of the fungus on wheat seed at temperatures of 70° to 75°C and treatment times of 150 to 180 s, when the initial seed moisture content was 15%. Using RF/V treatment, Dwinell and Carr[22] reported that no live nematodes were recovered from chips heated to 70°C. Nematodes were eradicated in boards when wood temperatures exceeded 48°C.

This study evaluated the effectiveness of RF treatment for the control of wood decay fungi (*Gloeophyllum trabeum, Ganoderma lucidum* (Leysser) Karsten, and *Irpex lacteus* Fries), and sapstain fungus (*Ceratocystis fimbriata* Ellis and Halsted) in red oak, poplar, and southern yellow pine wood species. A preliminary report has been published.[26]

Materials and methods

Wood block heating protocol

Ten blocks of each wood species were chosen to test heating uniformity and the heating rate required to reach the target temperature of 65°C (60°–70°C) in an industrial 40-kW dielectric oven. Target temperatures were chosen based on previous studies.[22,24,27] During RF heating, wood sample temperatures were monitored using ten fiber-optic electrode probes (Fiso, Quebec, Canada). To position the probes in the wood, small holes were drilled and the probes were placed tightly into the holes to obtain an accurate value. The moisture content (MC) was obtained from 21 wood blocks of each wood species. The initial weight was determined before RF treatment and was monitored daily to obtain constant MC.

Fungi

Four wood-destroying fungi: *Irpex lacteus*, *Ganoderma lucidum*, and *Gloeophyllum trabeum* (decay fungi), and *Ceratocystis fimbriata* (sapstain fungus) were chosen for this study because of their status as, or similarity to, species of concern from the Uinited States Department of Agriculture (USDA) pest risk assessment.[28] These fungi were grown on potato dextrose agar (PDA) or malt yeast extract agar (MYEA) for 2 weeks at 25°C or until almost complete colonization of the plates.

Wood species and inoculation

Red oak (*Quercus* spp.), poplar (*Populus alba* L.), and southern yellow pine (*Pinus* spp.) wood species were chosen for this study and used in all experiments. Blocks of red oak, poplar, and southern yellow pine (15.5 × 10 × 10 cm) were selected so that their thickness coincided with the radial direction of wood, and they were free of visible surface imperfections such as checking, knots, concentration of resins, or evidence of infection by mold, stain, or wood-destroying fungi (Table 1). Southern yellow pine appeared to have the highest degree of MC uniformity among the species examined. Approximately 1 g of macerated mycelium/spore mixture and 200 ml of sterile distilled water were blended in a Waring blender for 2 min before being poured into an autoclaved stainless-steel tray.

Wood samples were inoculated with the test fungi by dipping them to a depth of one surface deep (2 cm) into the inoculum. Identical red oak and poplar wood samples were not inoculated and served as controls. Samples were then incubated at 25°C for a minimum of 30 days. The untreated or uninoculated wood samples were used to detect background contamination (negative controls) and identical wood samples were inoculated with these pathogens but were not RF heated (positive controls). Wood samples at the time of inoculation had an average MC of 100% (red oak and poplar) and 16.1% (southern yellow pine) when tested at 50 mm by an electrical resistance moisture meter, which was confirmed using selected samples by oven-dried weight (Table 1).

The efficacy of the RF treatment in killing the wood decay fungi in wood blocks was determined by the ability (or lack thereof) to isolate the pathogen from the samples. Wood blocks were sampled by drilling through uninoculated or inoculated wood surfaces. Precautions were observed during drilling of wood blocks to prevent cross-contamination of samples. A biosafety hood and gloves were used during drilling and the working environment was swabbed with 70% alcohol for surface sterilization and to collect any bits of sawdust before and after drilling each sample. A rechargeable cordless drill was used. Drill bits were carefully cleaned with 5% NaOCl, rinsed for 2 min in sterile distilled water, soaked in 95% alcohol, and flame sterilized.

Drilled samples obtained at ten different locations on wood block surfaces were quickly transferred using flame-sterilized tweezers onto amended malt yeast agar providing a total of 10 isolations per block (a total of 100 isolation attempts per treatment). The agar contained 4 ppm benomyl to prevent the growth of unwanted mold, and 100 ppm tetracycline to inhibit the growth of bacteria. All isolations of suspected test fungi were subcultured onto PDA and subsequently compared with the reference test fungi used as controls. Pathogen isolation attempts were made prior to and after RF treatment. Positive and negative controls were treated in the same manner. Ten wood blocks were considered as a replicate and the experiment was conducted twice.

Results

Initial and preparatory experiments with 21 wood blocks of red oak, poplar, and southern yellow pine used to test the uniformity of the electromagnetic field revealed that the center of the electromagnetic field within the wood blocks was relatively uniform and that the temperature patterns after heating the wood blocks were reproducible (data not shown). The fungi, identified as *Gloeophyllum trabeum*, *Irpex lacteus*, *Ganoderma lucidum*, and *Ceratocystis fimbriata*, were reisolated from inoculated controls and in 2% of RF-treated samples. These fungi did not differ morphologically (based on light microscopy) or in their growth characteristics on MYEA medium when compared with the reference pathogens *G. trabeum*, *I. lacteus*, *G. lucidum*, and *C. fimbriata* used as controls.

Microscopic examination of test fungi from inoculated wood species confirmed the presence of *G. trabeum*, *I. lacteus*, *G. lucidum*, and *C. fimbriata* originally used as inoculum. No other fungal or bacterial contamination was observed during experimentation.

MC varied among wood species and was ≥ 100% in red oak and poplar prior to RF treatment and 84% on average after treatment. MC in southern yellow pine was 16.1% and 14.8% before and after RF treatments, respectively (Tables 1, 2). Overall, variation in MC was greater in red oak than in poplar or southern yellow pine.

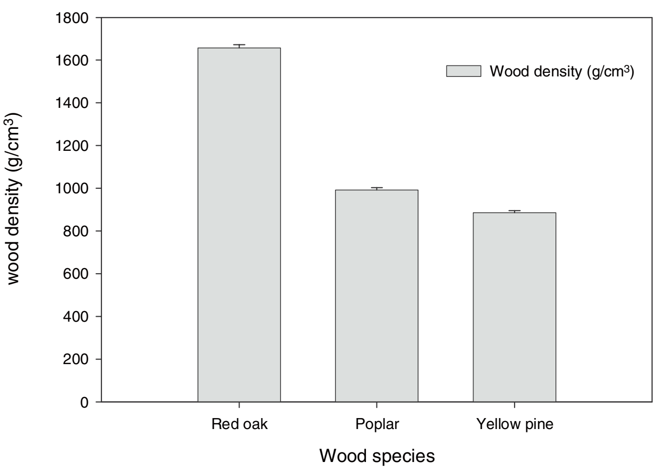

Fig. 1. Final wood density (g/cm^3) of red oak, poplar, and southern yellow pine after radio frequency treatment (21 wood blocks per species)

Results for density of wood species used in this study are shown in Fig. 1. The results showed that the density of red oak blocks was greater than that of poplar and southern yellow pine. The densities of poplar and southern yellow pine were not significantly different.

The relationship between heating time and temperature was linear, and the variation in heating time was longer when the average heating period was longer. The coefficients of determination of the regression of temperature as a function of heating time (period) were 80%, 81%, and 82% in red oak, poplar, and southern yellow pine samples, respectively. The target temperature of 65°C was achieved in most samples in approximately 2 min depending on the wood species and the MC (Fig. 2).

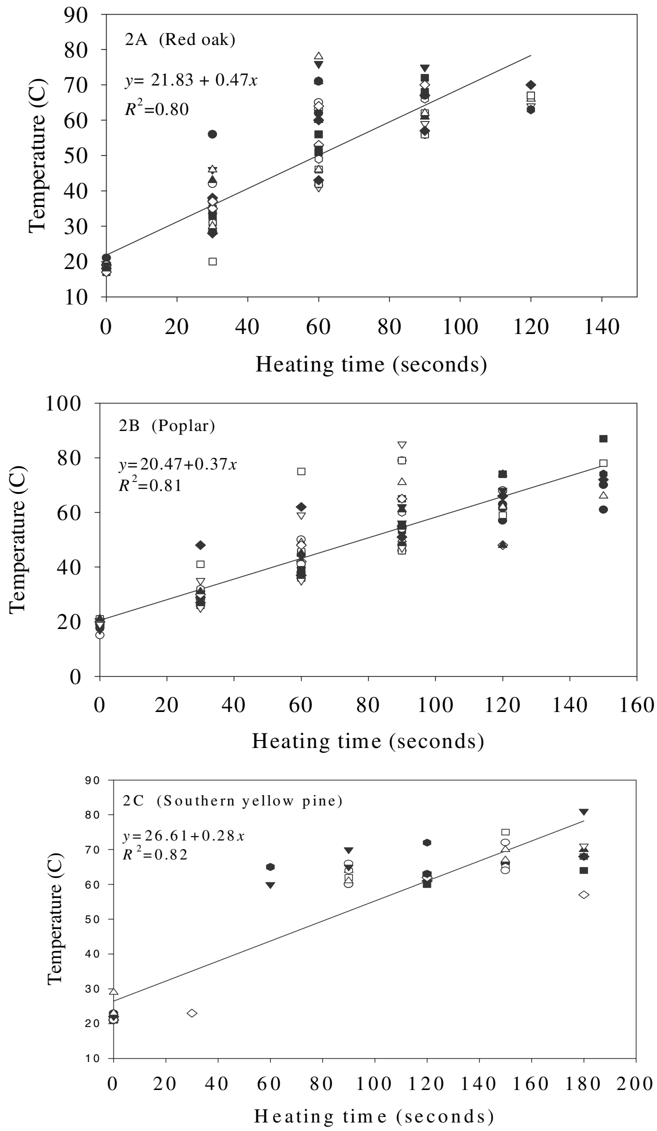

Fig. 2A–C. Time required to heat a wood block of **A** red oak, **B** poplar, and **C** southern yellow pine to target temperature of 60°–70°C with radio frequency energy. Temperatures were monitored using fiber-optic probes. Symbol (*filled squares, open squares, triangles, diamonds, open circles, filled circles*) represents temperature of wood samples when exposed to 40-kW radio frequency heating

Table 2. Moisture content of wood after heating, and heating characteristics of wood blocks infected with sapstain and wood decay fungi

Wood	Treatment	Final moisture content[a] (%)	Time[b] (min)	Heating rate (°C/s)	Power[c] (amps)	Electrode height (cm)
Red oak	Control	89.1				
	RF treated	84.6	1.27	38.5	0.84	20.3
Poplar	Control	83.2				
	RF treated	80.5	2.28	29.6	0.69	24.1
Yellow pine	Control	14.8				
	RF treated	14.8	2.15	22.1	0.69	11.4

[a] Final moisture content after drying wood block in oven set at 60°C for 48 h
[b] Time required to reach the target temperature
[c] Initial power

Table 3. Isolation of *Goeophyllum trabeum* from infected wood surface of red oak, poplar, and southern yellow pine before and after exposure to RF radiation at 60°–70°C in an industrial 40-kW dielectric oven

Wood species	Treatment	Pathogen isolation[a] (%)	
		Before	After
Red oak	Control	100	100
	RF treated	100	3
Poplar	Control	100	99
	RF treated	100	0
Southern yellow pine	Control	100	99
	RF treated	100	0

[a] Each percentage is the number of positive cultures out of 100 wood surface isolation attempts (10 samples each from 10 wood blocks per treatment)

Table 4. Isolation of *Irpex lacteus* from infected wood surface of red oak, poplar, and southern yellow pine before and after exposure to RF radiation at 60°–70°C in an industrial 40-kW dielectric oven

Wood species	Treatment	Pathogen isolation (%)	
		Before	After
Red oak	Control	100	100
	RF treated	100	2
Poplar	Control	100	100
	RF treated	100	0
Southern yellow pine	Control	100	100
	RF treated	100	0

Table 5. Isolation of *Ganoderma lucidum* from infected wood surface of red oak, poplar, and southern yellow pine before and after exposure to RF radiation at 60°–70°C in an industrial 40-kW dielectric oven

Wood species	Treatment	Pathogen isolation (%)	
		Before	After
Red oak	Control	100	100
	RF treated	100	3
Poplar	Control	100	99
	RF treated	100	0
Southern yellow pine	Control	100	100
	RF treated	100	0

In most cases, the temperature at the completion of a heating cycle exceeded the required temperature. The temperature was high in wood with high MC (red oak, 38.5°C/min) and low in southern yellow pine (22.1°C/min). Heating rates were relatively slow because of the low MC of wood samples. For example, the heating rate was 22.1°C/min in southern yellow pine with MC of 14.8%, and 38.5°C/min for red oak with MC of 84.6% (Table 2). MC loss was in the range of 76.4%–98.4% for red oak, 74.0%–85.0% for poplar, and 9.8%–14.6% for southern yellow pine. At a constant average heating temperature of 85°C, the MC of the red oak wood varied tremendously from 50% to 80%. In the case of southern yellow pine, the MC was more or less constant (10%–15%) at heating temperatures of 65°–75°C. The coefficients of determination of the regression equation were very low ($y = 66.12 + 0.10x$; $r^2 = 11\%$) for southern yellow pine and for red oak ($y = 0.85 - 0.16x$; $r^2 = 12\%$).

Reisolation results showed that *G. trabeum* was recovered from 99%–100% of untreated control wood samples, whereas none was recovered from RF-treated wood samples (100% inhibition) when the target temperature was applied (Table 3). *Gloeophyllum trabeum* was recovered in less than 3% of RF-treated red oak samples. In these samples the target temperature was not achieved (Table 3). The fungus *I. lacteus* was recovered from 100% of untreated controls, whereas none was recovered from RF-treated poplar and southern yellow pine samples when the target temperature was applied. *Irpex lacteus* was recovered in 100% of control samples and 2% of RF-treated red oak samples with high MC (Table 4). In these samples the target temperature was not reached.

Ganoderma lucidum was recovered from 99%–100% of untreated controls, whereas none was recovered from RF-treated poplar and southern yellow pine samples when the target temperature was applied. *Ganoderma lucidum* was recovered in 100% of control samples and 3% of RF-treated red oak samples (Table 5). *Ceratocystis fimbriata* was recovered from 99%–100% of untreated controls, whereas none was recovered from RF-treated poplar and southern yellow pine samples when the target temperature was applied. *Ceratocystis fimbriata* was recovered in 99% of control samples and 1% of RF-treated red oak samples (Table 6).

Table 6. Isolation of *Ceratocystis fimbriata* from infected wood surface of red oak, poplar, and southern yellow pine before and after exposure to RF radiation at 60°–70°C in an industrial 40-kW dielectric oven

Wood species	Treatment	Pathogen isolation	
		Before	After
Red oak	Control	100	99
	RF treated	100	1
Poplar	Control	100	100
	RF treated	100	0
Southern yellow pine	Control	100	100
	RF treated	100	0

Discussion

This study showed that 2 min of RF heating resulted in 98%–100% eradication of test or experimental decay fungi (*Gloeophyllum trabeum*, *Ganoderma lucidum*, and *Irpex lacteus*) and sapstain fungus (*Ceratocystis fimbriata*) in red oak, poplar, and southern yellow pine wood species. The isolation of fungi from wood samples previously inoculated and colonized by these specific pathogens, and the absence of pathogens in uninoculated control and RF-treated wood samples indicates that RF treatment can be an effective control against quarantined pests of certain commodities. The effectiveness of RF treatment on long-term decay of wood samples infected with the fungi over an extended period of time is not known.

After RF treatment, an odor was emitted from southern yellow pine wood. However, the wood samples showed no sign of physical damage, collapse, discoloration, or detectable internal stresses. It is questionable if this is a consequence of using small wood sections (15.5 × 10 × 10 cm), or if the results will vary when lumber of greater diameter and full length are exposed to RF treatment for similar or longer durations.

A considerable amount of past research has shown that density, MC, temperature, frequency, and grain direction have a major effects on the radio frequency properties of wood.[7–14,29,30] In general, we observed that increased heating time under our operating conditions resulted in increased wood sample temperatures, and, thus, increased fungal mortality. It is assumed that during RF treatment, the wood samples are heated in a short period of time and the heat is generated throughout the wood, allowing the temperature to rise faster than it would with conventional heat sterilization.[24] This process is superior to conventional kiln drying, which requires a considerable amount of energy,[31] a major portion of which is released into the atmosphere through ventilation. In our study, the effectiveness of RF treatment was demonstrated by low recovery of the fungus (high mortality) after exposure to RF radiation.

Results from density measurements reinforce the assumption that heavy wood blocks such as red oak will heat faster than lighter wood blocks such as poplar and southern yellow pine. It can be concluded that increase in MC in wood will result in a larger increase in actual wood weight we observed among wood species within the test conditions applied to this study. Dwinell et al.,[18] James,[29] Lin,[32] and Torgovnikov[30] working in other pathosystems reported on the internal heating and vapor generation from different woods with broad variation in density. Their findings corroborate our finding that red oak heats faster than poplar or southern yellow pine.

MC may also be an important factor to consider with RF treatments. These results demonstrate the importance of controlling the wood block MC to ensure uniform RF heating. Previous research has shown that MC is critical for effective RF and other heat treatments.[32] The relationship between heating time and temperature is dependent on wood MC (Fig. 2). The heating time is defined as the time it takes to heat the wood sample from room temperature to target temperature. A regression equation of heating temperature with time of exposure of wood samples (Fig. 2) indicates how well the linear model fits the mean experimental data. Therefore, this relationship may be used to predict the heating temperature requirement of wood samples based on the heating time period. The positive slopes of the regression equation are indicative of increasing temperature at greater time of exposure of wood samples to RF radiation. In our study, the influence of the heating time on temperature was more pronounced at higher MC. This implies that for effective treatment of wood samples with RF radiation, the MC of wood samples should be maintained at high levels (above 70%). Our results are corroborated by a previous study by Lin.[32] He reported that the effects of temperature and frequency on wood dielectric properties were more pronounced at high MC. This was attributed to a loss factor and a relaxation spectrum that shifted toward higher frequency when the temperature increased. This shift is caused by changes in the mobility of polar molecules with temperature.

In this study, eradication of the fungi by RF treatment depended strongly on initial wood moisture and the power density used. Wood moisture varied among species used in our studies, especially within red oak wood samples. This finding is in agreement with observations reported by Harris and Taras,[33] who reported red oak and hem fir to be highly variable in permeability and MC.

The study found that the eradication of the fungi by RF treatment depended on the temperature and the heating time. In other pathosystems such as *Bursaphalenchus xylophilus* (Steiner and Buhrer) and southern yellow pine,[4,5,22] nematode mortality depended on time and temperature.

For the three wood decay fungi and one sapstain fungus monitored in the present study, complete inhibition was observed in poplar and southern yellow pine subjected to RF treatment, whereas fungi were observed in less than 3% of RF-treated wood samples of red oak. This recovery might be due to high MC in the red oak at the time of RF treatment, and that the target temperature was not achieved in these samples. These results show that the RF treatment can, therefore, potentially provide an effective and rapid quarantine treatment as an alternative to MB fumigation for certain pathogen–wood combinations.

Additional research be conducted to determine the effects of MC, temperature, and treatment durations on pathogen inhibition or destruction, as well as treatment effects on other wood-inhabiting types of decay and sapstain fungi.

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