

# A Serendipitous Field Test against the Cellar Fungus *Coniophora olivacea*

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## Abstract

A weather-protected aboveground field test was established at Kincardine, Ontario, Canada, to assess the performance against termites of two formulations of carbon-based preservative in Pacific silver fir (*Abies amabilis*) and white spruce (*Picea glauca*). A naturally durable untreated reference species was also included. The boards were visually inspected for decay and termite attack after 6 years. Termite attack was negligible, but the test material was challenged by *Coniophora olivacea*, a soil-inhabiting, strand-forming, brown-rot fungus. While decay was well advanced in some untreated controls and western red cedar (*Thuja plicata*) reference samples, treated samples that had been similarly challenged remained in excellent condition.

The wood preservation industry continues to evolve with the introduction of new preservative formulations, for which Canada's Pest Management Regulatory Agency requires in-service efficacy data to allow registration. In 2007, an aboveground termite exposure test of two formulations of a carbon-based preservative was set up at Kincardine, Ontario, Canada, which turned out to be totally unsuccessful as a termite test but very successful as a test of resistance to the cellar fungus *Coniophora olivacea* (Fr.) P. Karst. This article describes the test results after 6 years of exposure.

## Materials and Methods

Eight-foot 2 by 6-inch kiln-dried boards containing a mix of sapwood and heartwood were obtained for three species: Pacific silver fir (*Abies amabilis* Dougl. Forbes), white spruce (*Picea glauca* (Moench) Voss), and western red cedar (*Thuja plicata* Donn). The western red cedar was intended as a reference naturally durable material in this test. Half of the white spruce boards were incised prior to treatment. The more permeable Pacific silver fir boards were end sealed with two coats of epoxy resin (Interguard 740, International Paints). The test boards were pressure treated with two formulations of carbon-based preservative, one called PTQ (propiconazole, tebuconazole, quat) and the other called formulation C, using solution strengths listed in Table 1. The treating schedule consisted of an initial 30-minute vacuum at 22 inches Hg, followed by 60 minutes at a pressure of 150 psi, and a final 15 minutes of vacuum.

After air-drying, one 400-mm sample for this test was cut from the center of each of the 10 selected boards, plus an

adjacent biscuit for determination of penetration and retention. Both of the cut ends of the 400-mm-long experimental samples were brush coated with two applications of zinc naphthenate (2% Zn) field-cut preservative. The samples were labeled on the sapwood face with stainless steel labels. All samples were then stickered to air-dry at ambient conditions for 1 month. After oven-drying overnight at  $103^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , the biscuit was cut into subsamples. One subsample was used for preservative penetration measurement on the face and edge of the piece; then the percentages of the 10 samples per group meeting the 5-mm and 10-mm penetration requirements were calculated. For formulations C and PTQ, borate and didecyl dimethyl ammonium carbonate (DDACarb) indicators, respectively, were used to measure penetration. The other subsample was used for preservative analysis. Sawdust from a 13-mm-edge assay zone for each replicate was sent to Arch Wood Protection in Conley, Georgia, for analysis (American Wood Protection Association [AWPA] 2007a, 2007b, 2007c).

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Table 1.—Solution strengths.

Species <sup>a</sup>	Incised	Formulation <sup>b</sup>	Target retention	Solution strengths for target retentions				
				%Teb/Prop <sup>c</sup>	Kathon WT (ppm)	Acticide 45 (ppm)	Borax (%)	DDAC (%) <sup>d</sup>
PSF	No	C	Medium	0.053	40	150	0.3	N/A
PSF	No	PTQ	High	0.078	40	N/A	N/A	0.6
WS	No	C	High	0.247	40	150	0.3	N/A
WS	Yes	C	Medium	0.114	40	150	0.3	N/A
WS	No	PTQ	High	0.350	40	N/A	N/A	0.6
WS	Yes	PTQ	High	0.165	40	N/A	N/A	0.6

<sup>a</sup> PSF = Pacific silver fir; WS = white spruce.

<sup>b</sup> C = formulation C; PTQ = propiconazole, tebuconazole, quat.

<sup>c</sup> Teb/Prop = tebuconazole/propiconazole.

<sup>d</sup> DDAC = didecyl dimethyl ammonium carbonate.

The test method was as described in AWWA Standard E21-07 (AWWA 2007d). This method simulates a sill plate on a concrete foundation that falls within Use Category 2 in the AWWA standard (AWWA 2012). Ten boxes were constructed from white painted chromated copper arsenate-treated plywood, 600 mm wide by 350 mm high by 1,000 mm long with an open bottom. The test site is located in Kincardine, Ontario. The soil is a very well-drained sandy loam. The predominant type of decay at this test site is from brown-rot basidiomycetes, including *Coniophora arida* (Fr.) P. Karst, *C. olivacea*, *Gloeophyllum sepiarium* (Wulfen) P. Karst, and *Gloeophyllum trabeum* (Pers.) Murrill. This site receives mean annual precipitation of 998 mm and has mean daily maximum and minimum temperatures of  $-2^{\circ}\text{C}$  and  $-10^{\circ}\text{C}$  in January and  $24^{\circ}\text{C}$  and  $13^{\circ}\text{C}$  in July, with an average yearly temperature of  $6.2^{\circ}\text{C}$ . The climate there also places it within the zone of medium out-of-ground decay hazard with an updated climate index of 49 (value for Owen Sound; Morris and Wang 2008). This test plot also has a population of the subterranean termite *Reticulitermes flavipes* Kollar.

For each test array, eight hollow masonry concrete blocks were placed onto leveled soil in a 4 by 2 array, 50 mm apart. Through the two holes in each block, 25 by 25 by 300-mm pine heartwood feeder stakes were hammered into the ground so the top of the stake was within 2 to 5 mm of the top of the block. The feeder stakes, which were slightly durable ponderosa pine (*Pinus ponderosa* Laws) heartwood, were installed with the intention that they would remain free from decay and mold long enough to be discovered by termites. The test samples were installed such that the samples cover the holes in the block but are not in direct contact with the feeder stake. This was to prevent direct

tunneling by termites from the untreated wood stakes into the test samples.

In each test array, one sample from each group was installed on top of each of the blocks, and the test arrays were covered with the boxes. In August 2013, each sample was removed from the covering box and examined visually for termite attack decay. Each specimen was then assigned a rating, based on the AWWA E21 grading system for decay (Table 2). One feeder stake from this test was sampled to isolate the fungus present. The fungus was isolated from mycelium onto 1 percent malt extract agar (MEA) amended with 20 ppm of Benomyl 50 fungicide. Pure subcultures were made onto 1 percent MEA, and the DNA was extracted from this culture and also directly from the mycelia collected off of two other feeder stakes using the Qiagen DNeasy Plant Minikit (Qiagen, Germany). DNA was amplified for sequencing by polymerase chain reaction using the basidiomycete ribosomal DNA primers ITS-F and ITS4-B (Gardes and Bruns 1993). Samples were sequenced at the Plateforme de Séquençage et de Génomique des Génomes, Centre de Recherche du CHUL (Quebec, Canada).

## Results and Discussion

At the 6-year inspection, termite attack was negligible; however, in 7 of the 10 boxes, a basidiomycete from the soil with strands characteristic of *Coniophora* spp. had challenged 56 of the 80 samples via the feeder stakes. DNA from the fungus on all three feeder stakes sampled was a 98 to 99 percent match to *C. olivacea* (Fr.) P. Karst sequences in the National Center for Biotechnology Information database (83% to 91% query coverage of a 700-base-pair fragment). This is one of a group of *Coniophora* species known as the cellar fungi (Schmidt et al. 2005). Only one of

Table 2.—American Wood Protection Association rating system.

Decay rating	Condition	Description
10	Sound	No sign or evidence of decay, wood softening, or discoloration caused by microorganism attack
9.5	Trace suspect	Some areas of discoloration and/or softening associated with superficial microorganism attack
9	Slight attack	Decay and wood softening is present; up to 3% of the cross-sectional area affected
8	Moderate attack	Similar to 9 but more extensive attack with 3% to 10% of cross-sectional area affected
7	Moderate/severe attack	Sample has between 10% and 30% of cross-sectional area decayed
6	Severe attack	Sample has between 30% and 50% of cross-sectional area decayed
4	Very severe attack	Sample has between 50% and 75% of cross-sectional area decayed
0	Failure	Sample has functionally failed; it can either be broken by hand due to decay, or the evaluation probe can penetrate through the sample

Table 3.—Penetration and retention in treated boards with means and standard deviations.

Formulation <sup>a</sup>	Species <sup>b</sup>	Target	Incised	Penetration				Assay retention, mean (SD) (kg/m <sup>3</sup> )	Mean (SD) rating	
				Heartwood face		Edge			10 reps	7 fungus-challenged reps
				% ≥5 mm	% ≥10 mm	% ≥5 mm	% ≥10 mm			
None	PSF	N/A	No					8.0 (3.5)	7.1 (4.0)	
C	PSF	Medium	No	100	90	100	60	0.46 (0.18)	9.9 (0.3)	9.9 (0.4)
C	WS	High	No	50	10	50	10	0.12 (0.06)	10.0 (0.0)	10.0 (0.0)
C	WS	High	Yes	100	0	70	0	0.30 (0.12)	10.0 (0.0)	10.0 (0.0)
PTQ	PSF	Medium	No	40	10	40	20	0.81 (0.43)	10.0 (0.0)	10.0 (0.0)
PTQ	WS	High	No	0	0	10	0	0.31 (0.37)	10.0 (0.0)	10.0 (0.0)
PTQ	WS	High	Yes	50	0	90	10	0.67 (0.17)	10.0 (0.0)	10.0 (0.0)
None	WRC	N/A	No					8.5 (2.3)	7.9 (2.5)	

<sup>a</sup> C = formulation C; PTQ = propiconazole, tebuconazole, quat.

<sup>b</sup> PSF = Pacific silver fir; WS = white spruce; WRC = western red cedar.

the treated samples challenged by this fungus showed early decay. This was a formulation C–treated Pacific silver fir sample, rated 9. All of the PTQ samples were sound, with a mean rating of 10 (Table 3). In three of seven of both the Pacific silver fir controls and western red cedar reference samples challenged by this fungus, decay was moderate/severe, with one failure in Pacific silver fir. An additional western red cedar untreated sample was rated 9 for early attack. The similar level of attack on challenged controls (mean, 7.1) and western red cedar (mean, 7.9) in this test suggests that this *Coniophora* strain is resistant to western red cedar extractives.

### Conclusions

After 6 years’ exposure, untreated control boards and western red cedar reference samples were severely attacked by *C. olivacea*, a strand-forming, wood-rotting basidiomycete, while the PTQ-treated samples challenged by the same fungus remained in excellent condition.

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