Unique Preservative Tolerance in *Tyromyces palustris*

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Fungal tolerance to wood preservatives has been critically important for many years. The tolerance of type cultures to common wood preservatives is, in fact, noted in the AWPA Standards [1]. *Gloeophyllum trabeum* Mad-617, for example, is particularly tolerant to phenolic and arsenic compounds. One important group of decay fungi that has been studied extensively are those that are copper tolerant such as *Postia placenta* MAD-698, which is particularly tolerant to copper and zinc compounds [1]. Recently, *Tyromyces palustris* and *Serpula lacrymans,* the European dry rot fungus, have also been shown to be copper tolerant [2,3,4].

Replacement of CCA-treated wood from residential markets in the USA with copper-based organic systems has renewed concerns about the decay capacity of copper-tolerant fungi as copper remains the primary component of the new generation of preservatives. In a series of papers on copper tolerance; *Tyromyces palustris* TYP 6137 produced high levels of oxalic acid (581mM) on untreated southern yellow pine (SYP) and 42% mass loss on copper citrate-treated SYP [2,5]. Oxalic acid (OA) has previously been implicated as a key fungal metabolite in precipitation and neutralization of copper by brown-rot fungi [6]. In a study of copper tolerance to arsenic-free preservatives (ACQ-D, NHA, CuBor) TYP 6137 and L-15755 produced nearly 10% weight loss on 0.5% NHA treated blocks but little or no weight loss on ACQ-D or CuBor [2]. In further studies of N'N naphthaloylhydroxylamine (NHA) leachability, TYP-6137 caused significantly higher weight loss of NHA-treated SYP and balsa than *Gloeophyllum trabeum* or *Meruliporia incrassata* [7]. *T. palustris* TYP 6137 also showed equal capacity to cause weight loss of 0.5% NHA-treated blocks and SYP controls (44.3+/-4.8 vs 48.2+/-6.4) [4]. We concluded from these observations that TYP 6137 and L-15755 show partial tolerance to NHA that is not observed in other common brown-rot fungi [4].

N'N naphthaloylhydroxylamine (Na-NHA: $C_{12}H_6NNaO_3$) is a naphthalene derivative, and a selective calcium precipitating compound [8]. NHA has recently been patented and licensed as a termite bait [9]. NHA tolerance is logically linked to the mechanism of NHA inhibition. Previous investigations into this mechanism have shown that the protonated form of NHA (H-NHA) is more inhibitory to decay fungi than the sodium form (eg Na-NHA)[9]. Due to excess OA production during incipient decay by *T. palustris,* we assume that most or all of the soluble Na-NHA will rapidly be converted into the acid form (H-NHA), at least initially. What type of subsequent decay event could then render the H-NHA inactive? We have theorized at least five possible mechanisms: i) translocation of calcium, iron or manganese into the decay site which would precipitate the H-NHA and replace the hydrogen atom, (e.g. Bech-Anderson noted that the dry rot fungus *S. lacrymans* required a calcium source of building materials to neutralize extracellular OA [11]); ii) cleavage by extracellular hydrolytic enzymes; iii) oxidative Fenton chemistry (one electron oxidation); iv) a barrier effect initiated by precipitation of NHA on or outside the hyphae sheath, e.g calcium oxalate is observed precipitated near or onto the hyphal sheath [12]; and iv) binding of carbonyl groups of NHA to carbohydrate wood components at very low pH (~1.0) by acid-catalyzed aldol condensation (personal communication) [13].

Experiments were designed to test the "calcium neutralization" hypothesis using CaCl and $CaCO_3$, but failed to produce a positive result. We also designed two experiments to determine if the NHA was "degraded" by *T. palustris* by secondary testing of *T. palustris* 6137 or L-15755 degraded wood blocks by *Reticulitermes flavipes* or non-NHA-tolerant wood decay fungi after the method of Duncan and Deverall [14]. These experiments showed that the NHA was still active in inhibition of termites and non-tolerant decay fungi. This result suggests that NHA is blocked from inhibitory effects of the proteinated form but not oxidatively or enzymatically degraded.

We conclude that TYP-6137 and TYP-L15755 are tolerant to higher levels of NHA compared to other brown-rot fungi by some heretofore undescribed ''barrier'' mechanism, possibly mediated by the hyphal sheath. Experiments to date have yet to confirm the precise mechanism of NHA tolerance.

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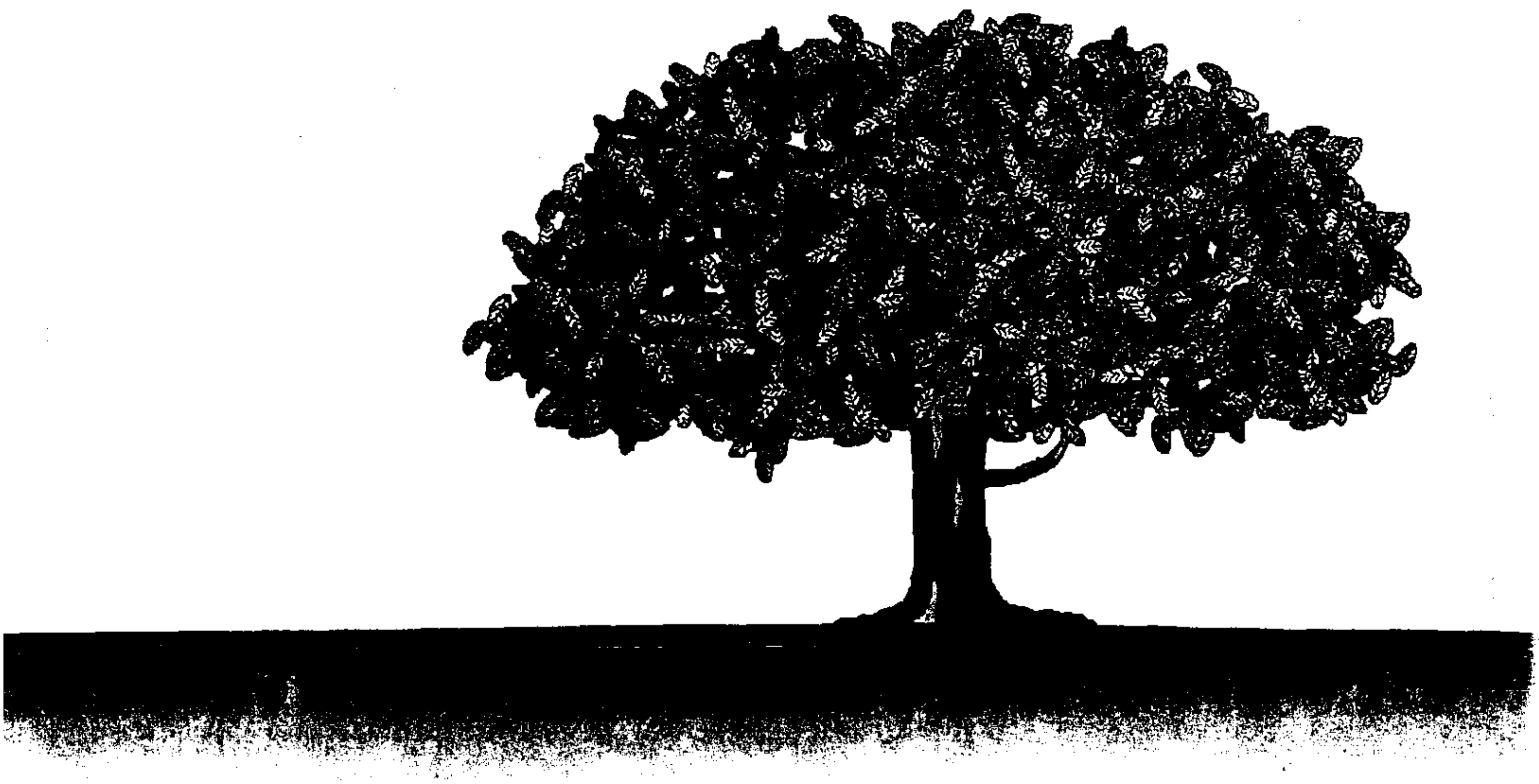

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