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QUANTIFYING PROPICONAZOLE IN WOOD BY RAMAN MICROSCOPY

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Summary

The Raman signature of propiconazole at 647-693 cm^{-1} region was used to determine the propiconazole distribution in white spruce. Samples treated with propiconazole were milled at ~1.5 mm intervals and analysed by methanol extraction and GC-MS to obtain a depth profile in the longitudinal direction. The concentration of propiconazole in the milled wood layers ranged from 0.7 to 3.4 mg/g (dry mass wood). The average Raman signal from each of the layers was linear with the GC-MS-determined concentrations. Raman microscopy also revealed that propiconazole was concentrated in the summer wood, and that the antifungal agent tended to bloom to the surface in the samples studied.

1. Introduction

Propiconazole is a fungistatic agent (Ciba-Geigy) that inhibits biosynthesis of ergosterol, an important constituent of fungal cell walls. The agent was first developed in 1979 by Janssen Pharmaceuticals of Belgium. Originally intended for pharmaceutical use, it is currently used for protecting crops and wood. The toxicity of propiconazole is low (EXTOXNET, 2001), making it a good environmental choice; however, determination of the depth of penetration is very time consuming. An *in situ* technique would require much less sample preparation and analysis time. The main objective of this work was to study the applicability of Raman microscopy to the quantitative determination of propiconazole in wood. A second objective was to determine whether the agent adsorbed preferentially to particular wood structures.

To our knowledge, there are no literature reports of using Raman to study propiconazole on wood. Raman has been used to study natural components of wood--for example, lignin (Agarwal, 1999; Ibrahim and Oldham, 1997; Wariishi et al., 1997) and carbohydrates (Ona et al., 1998; ibid. 1997; Agarwal and Ralph, 1997) and pinosylvins (Holmgren et al., 1999). Results of such studies have been combined with statistical methods to distinguish soft and hard woods (Lavine et al., 2001; Yang et al., 1999). Raman studies of wood bonded with resins, such as the diphenyl methane di-isocyanate (MDI) binders used in oriented strand board have also been reported (Yamauchi et al., 1999; *ibid.* 1997). The influence of capillary and non-capillary sections of pine on absorption of water, formamide

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and diiodomethane has also been investigated (Shen et al., 2001) with capillary sections showing much greater absorption of the liquids than the non-capillary sections. Cell types (fibre, ray parenchyma, and axial parenchyma) and softwood tissues can be distinguished on the basis of their Raman spectra (Ona et al., 1999; Bond et al., 1999).

The current work demonstrates that propiconazole is quantifiable by Raman microscopy. The Raman signature of propiconazole at 647-693 cm^{-1} region is free of interference from wood signals. The Raman wood signals about 985-1175 cm^{-1} serve as normalisation standards to correct for surface effects. In addition, the high spatial resolution of the Raman microscope reveals that propiconazole deposits preferentially in the summer wood.

2. Methodology

Sample Preparation and Extraction

Samples of white spruce and propiconazole (50% in mineral spirits) were obtained from EverDry Forrest Products Ltd. The spruce samples were cut with a band saw. Dimensions were 2.7 cm in the longitudinal direction, 2.6 cm approximately along the radial direction, and 9.8 cm in the tangential direction. The blocks were soaked in a 3% solution of propiconazole in mineral spirits (Manufacturer, product code) for 24, 48, or 96 hours. The samples were then removed from the solution and dried in fumehood at ambient temperature for 36 hours to remove all the mineral spirits (undetectable by GC-MS).

In order to obtain a longitudinal depth profile, the samples were ground using a milling machine. First, a thin sliver of sample was removed from the centre of the sample, with the long edge in the longitudinal direction. This was saved for Raman analysis. Layers approximately 1.5 mm thick (longitudinal direction) were milled from the remaining sample, and the ground wood from each layer was collected separately in a plastic bag. The ground wood was then screened at 40-mesh. The screened wood was weighed and extracted with 25 mL of methanol [Manufacturer, product code] for 4 hours in a shaker bath. The extracts were filtered (0.45 μm, PTFE) and added to a 25 mL flask**.** A standard amount of azaconazole was added to each flask, and the volume was made up to the mark with methanol.

GC-MS Determination of Propiconazole

The methanol solutions obtained were analysed by GC-MS (Perkin-Elmer) with a 95% methyl polysiloxane and 5% phenyl column (Manufacturer, product code). The injector was set at 250°C, and the column was ramped from 25°C/min to 350°C. Chromatograms were first recorded under total ion current mode, scanning from 50 to 350 amu in order to determine the best masses for observing azaconazole and propiconazole. Two isomers of propiconazole were fully resolved, with retention times of 9.26 and 9.31 minutes, in a ratio of about 1:2. Both peaks were integrated to obtain the total propiconazole signal. The azaconazole peak was observed at 8.80 minutes. The analytical mass peaks for propiconazole and azaconazole were 69 and 217 amu, respectively. A background-free

chromatogram was obtained by monitoring the signal at 217 amu, then switching to 69 amu at 9.00 minutes. Integration times were set to give at least 10 points across each of the three peaks.

Raman Spectra

Raman measurements were performed on the sliver of sample removed prior to grinding. Spectra were collected using a Renishaw System 2000, a dispersive system. The instrument was equipped with a Pelletier-cooled CCD array that could collect a 600 cm^{-1} spectral window (spectrograph) with 2.5 cm^{-1} spectral resolution and ~2 μm spatial resolution. Excitation was achieved with a 785-nm diode laser (30 mW). The long wavelength was necessary to reduce fluorescence, probably due to low-lying electronic states of extractives (tannins, e.g.). Spectra were obtained from the summer wood only, as it was found that the spring wood did not retain propiconazole. Spectra were collected from 2-4 points within the area corresponding to a given milled layer, and averaged. The spectral window from 600 to 1200 cm^{-1} (Raman shift) was integrated for 300 s with the cosmic ray elimination feature off (total collection time per spectrum ~10 min.).

3. Results and Discussion

GC-MS Determination of the Propiconazole Depth Profile

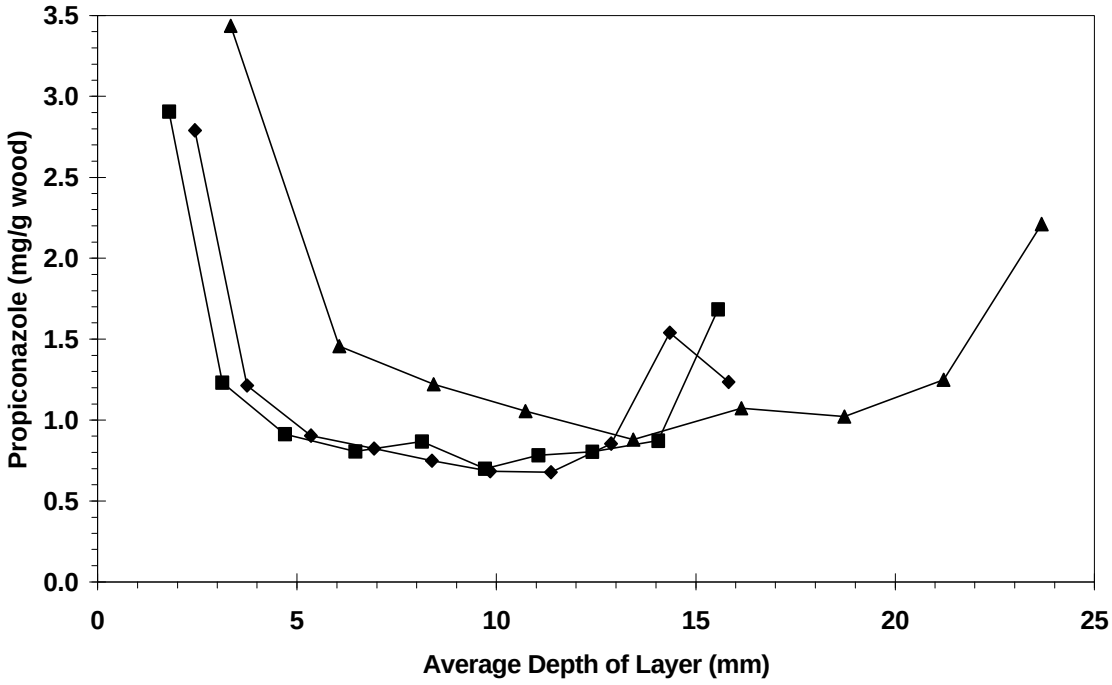

Figure 1: Depth profile of propiconazole in spruce samples soaked in 3% propiconazole. Soaking times: diamonds: 24 h; squares: 48 h; triangles: 96 h.

Figure 1 shows the depth profile of propiconazole along the longitudinal direction of the wood sample, as determined by GC-MS. The points represent the *average* concentration of propiconazole in each layer. The depth profiles of the three samples, soaked at 24, 48, and 96 hours, are nearly identical, indicating that the wood was saturated with the soaking solution prior to 24 hours. The profiles were all "bowl" shaped, the concentration decreasing from the edges to a rather flat minimum in the middle of the sample. The shape is consistent with a diffusion mechanism. Because the milling machine could not grind the sample down to the lower surface safely, only the top surface of the sample could be analysed. The concentration in the surface layer was much higher than that of the subsequent layers (35, 42, and 94 mg g^{-1} in the 24, 48, and 96-h samples, respectively). Raman analysis indicated that most of the propiconazole in that layer was located on the surface, suggesting that the propiconazole had "bloomed" to the surface as the sample dried. Because the Raman spectra were measured normal to the longitudinal direction, and so could not include the surface, the Raman intensities would not correlate with the GC-determined concentration of that layer. Consequently, the first point of each profile (the one that included the top surface) was eliminated from Figure 1.

Raman Spectra and Correlation with GC-MS Results

Fluorescence is arguably the biggest problem for Raman studies of complex matrices, such as native wood. The large, broad fluorescence signals are in the same spectral range as Raman shifts. The shot noise on fluorescence can be as large as the Raman peaks. Fortunately, fluorescence can be reduced by using a long-wavelength laser. The 785-nm laser used in these experiments is on the edge of the near infrared region, and so should have low efficiency for electronic excitation. In our preliminary experiments, we found that many species of wood exhibited a high fluorescence, even at 785 nm. There appeared to be a correlation with the colour of the wood; darker woods tended to have higher fluorescence than lighter woods. A survey of several species (red pine, white pine, southern pine, western red cedar, white spruce, red maple, and aspen) revealed that white spruce and aspen had the lowest fluorescence, red cedar the highest. Within a given sample, the heartwood tended to fluoresce higher than the sapwood (Figure 2). This observation implicates the dark-coloured extractives (for example, tannins) as the source of the fluorescence. While the current study has been restricted to white spruce, it is likely that Raman could be useful for analysing propiconazole in other species if an even longer wavelength is used (e.g. 1064 nm).

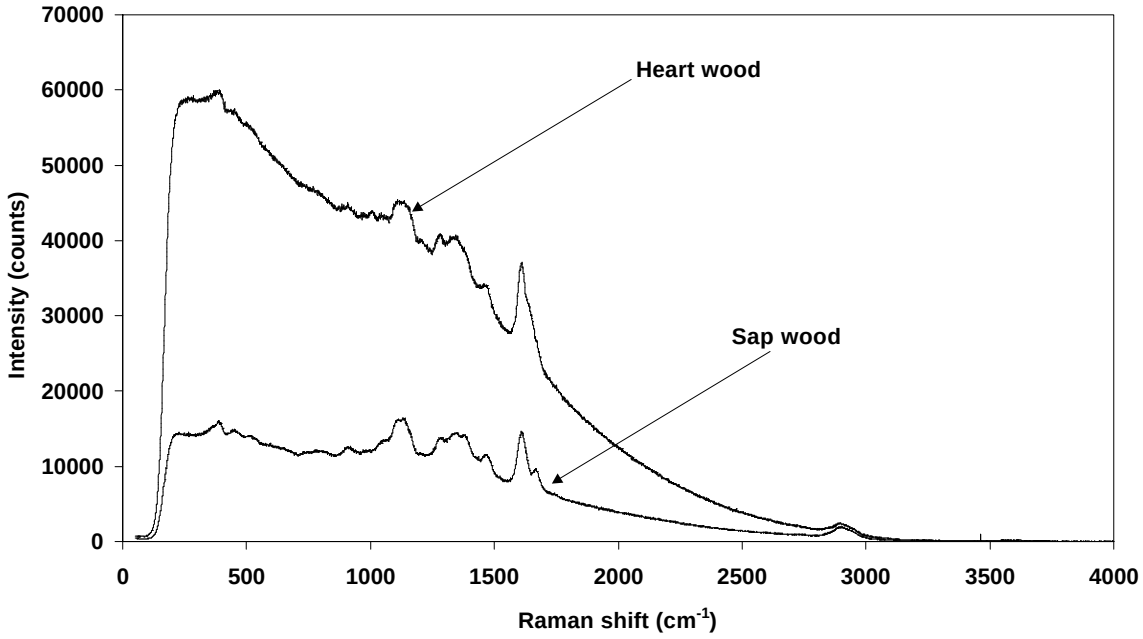

Figure 2: Raman spectra of Red Pine

Figure 3 shows the Raman spectra of spruce, spruce treated with propiconazole, and 99.7% propiconazole. The propiconazole Raman signal at 647-693 cm^{-1} was chosen as the analytical peak for its intensity and because it did not overlap the peaks of the wood spectrum. Absolute Raman signals vary with surface roughness. For samples such as wood, for which surface properties can vary enormously, quantitative determinations require a normalisation procedure. In this work, the analytical peak area was normalised by dividing by the area of the wood signals between 985-1175 cm^{-1}. In this way, the wood served as an internal standard for the propiconazole. Because the wood peaks in the range 985-1175 cm^{-1} overlapped with some propiconazole peaks, the wood signal had to be corrected before it could be used for normalisation by subtracting an estimate of the area due to propiconazole signal.
The normalised signals were calculated as:

$$\text{Normalised Intensity} = \frac{A_3}{A_4 - \frac{A_2}{A_1} A_3} = \left(\frac{A_4}{A_3} - \frac{A_2}{A_1} \right)^{-1}$$

where A_1 is the area under the 647-693 cm^{-1} region in the standard propiconazole spectrum, A_2 is the area under the region 985-1175 cm^{-1} in the standard propiconazole spectrum, A_3 is the area under the analytical region (647-693 cm^{-1}) in the spectrum of the treated wood, and A_4 is the area under the region 985-1175 cm^{-1} in the spectrum of treated wood.

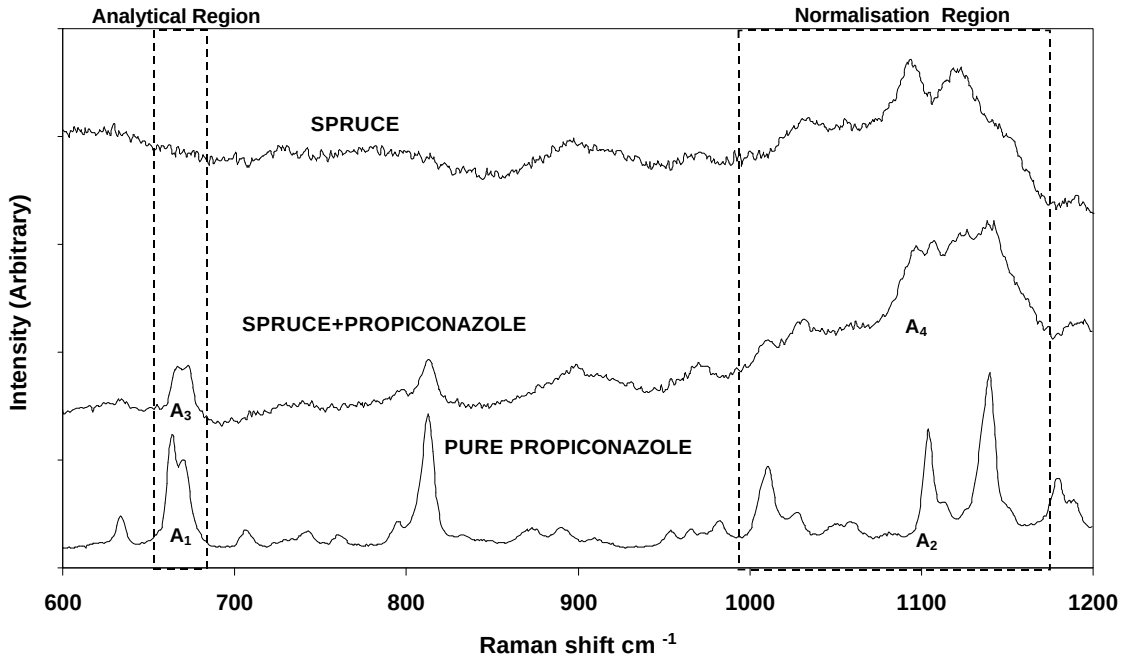

Figure 3: Raman spectra of spruce, spruce treated with 3% propiconazole, and propiconazole standard (Janssen, 99.7%)

The spatial resolution of Raman microscopy (~2 μm) is very suitable for studies of wood because the sizes of the structures in wood (channels, cells, and even cell walls) are larger than the resolving power of the microscope. The power of this microscopic technique is apparent in figure 4, which shows dramatic differences between spring wood and summer wood in the same ring of a sample. Repeated analysis has shown that propiconazole preferentially deposits in the summer wood. In fact, propiconazole was not observed in the spring wood in any of the samples studied. As previously mentioned, propiconazole blooms have been observed on the surface of the samples. The low levels of propiconazole in the spring wood may be due to the more open structure of the spring wood, which allows the solution to diffuse out easily compared to the denser summer wood. Because the concentration in spring wood was below the detection limits, all Raman spectra in this study were sampled from summer wood.

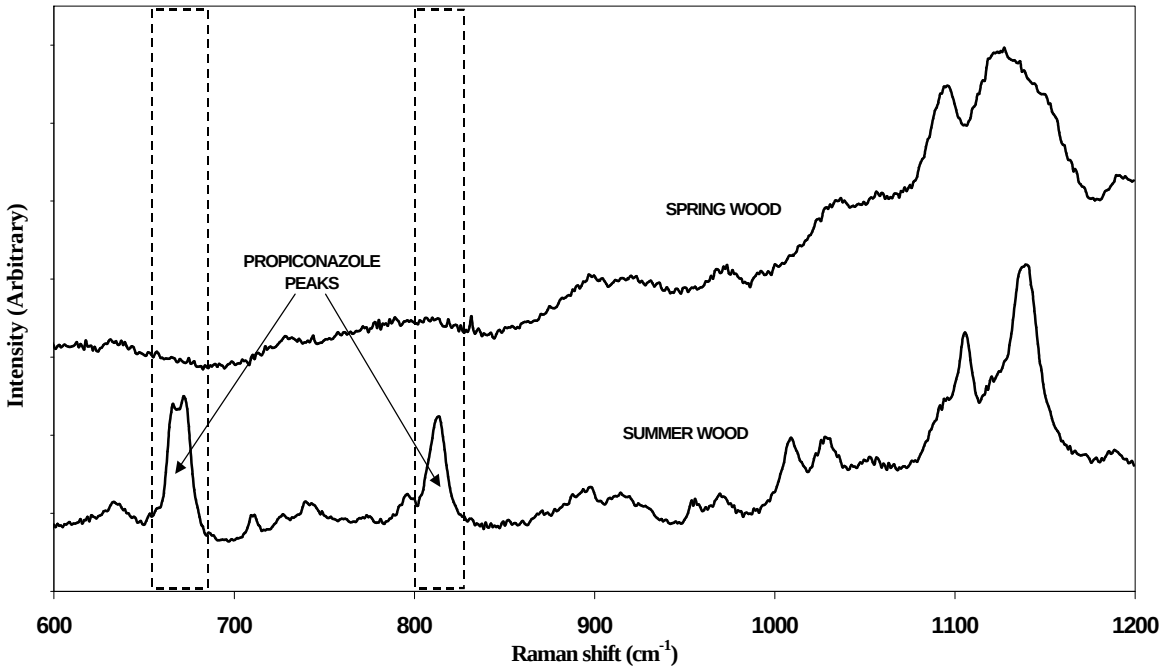

Figure 4: Raman spectra from spring and summer wood. Propiconazole signals are absent in the spring wood.

Figure 5 shows the average, normalised Raman intensity for the 48-hour and 96-hour samples, plotted against the average layer depth. (The 24-hour sample required further analysis at the time of writing.) The curves are qualitatively similar to the depth profiles obtained by GC-MS, although the data is more scattered than in the GC-MS curves. The larger standard deviations arise chiefly from the small sampling volume of the Raman microscope. The signal from the microscopic measurements comes from two sources. Firstly, the volume sampled by the Raman microscope is on the order of the cube of the focus of the laser, which was about 1 μm at the waist in these experiments. The GC data used a wood sample approximately 10^{10} times larger by volume, and so should be expected to produce better signal-to-noise. Second, and perhaps more importantly, the distribution of propiconazole was very inhomogeneous. As has been discussed, propiconazole was undetectable in spring wood, and it is likely that the concentration in summer wood was not uniform. The scatter in the data could be reduced by collecting more spectra or by using a sampling technique with a larger spot size (a fibre-optic probe, for example).

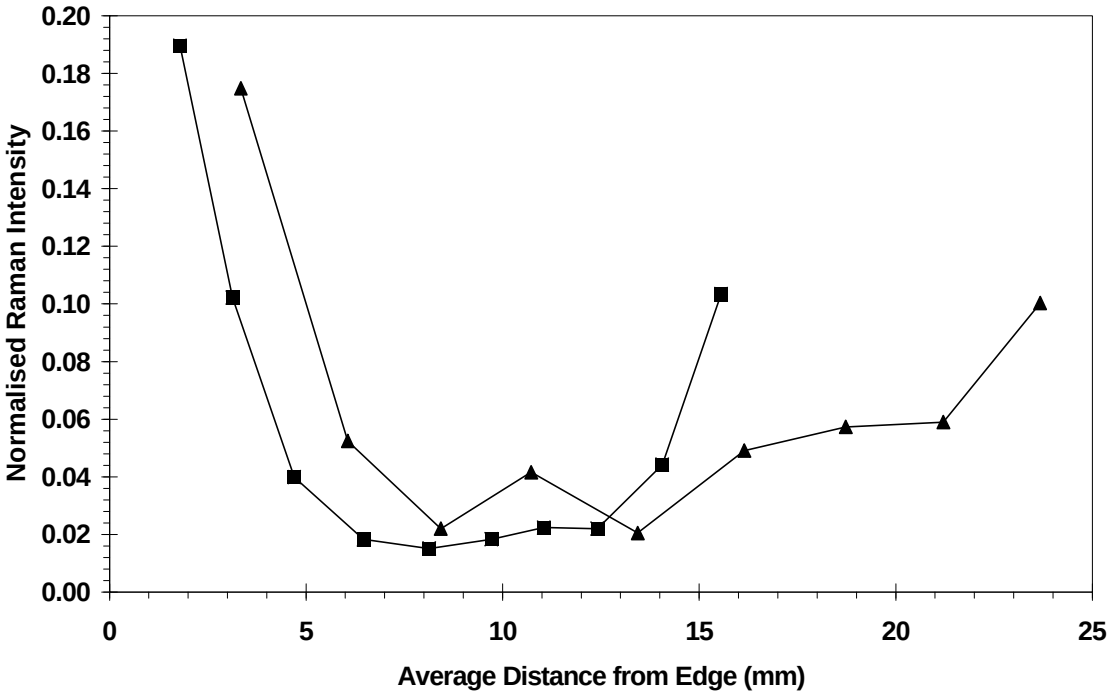

Figure 5: Raman depth profiles of treated spruce samples for the analytical peak (690 cm^{-1}) of propiconazole. Samples soaked in 3% propiconazole for: squares—48 hours, triangles—96 hours.

In figure 6 the Raman signals from the 48-hour and 96-hour samples analysed in figure 5 are plotted against the concentrations determined by GC-MS (figure 1). The data are from two samples prepared and analysed weeks apart, yet the results of both analyses are clustered about the same line. The curve is linear, with $R^2 = 0.865$. The low R^2 value puts the method in the "semiquantitative" range; however, there is good reason to believe that the method can be improved to make it more quantitative. First, the points are primarily clustered about the 1 mg g^{-1} region, due to the fairly constant values of propiconazole concentration in the interior of the samples. The curve could be improved by the addition of more points at other concentrations. Lower and higher values of propiconazole in wood can be obtained from samples soaked in solutions of lower and higher concentration. Second, as was previously mentioned, the scatter could also be improved by increasing the number of Raman spectra per layer. (For this study only 2 to 4 spectra were averaged per layer.)

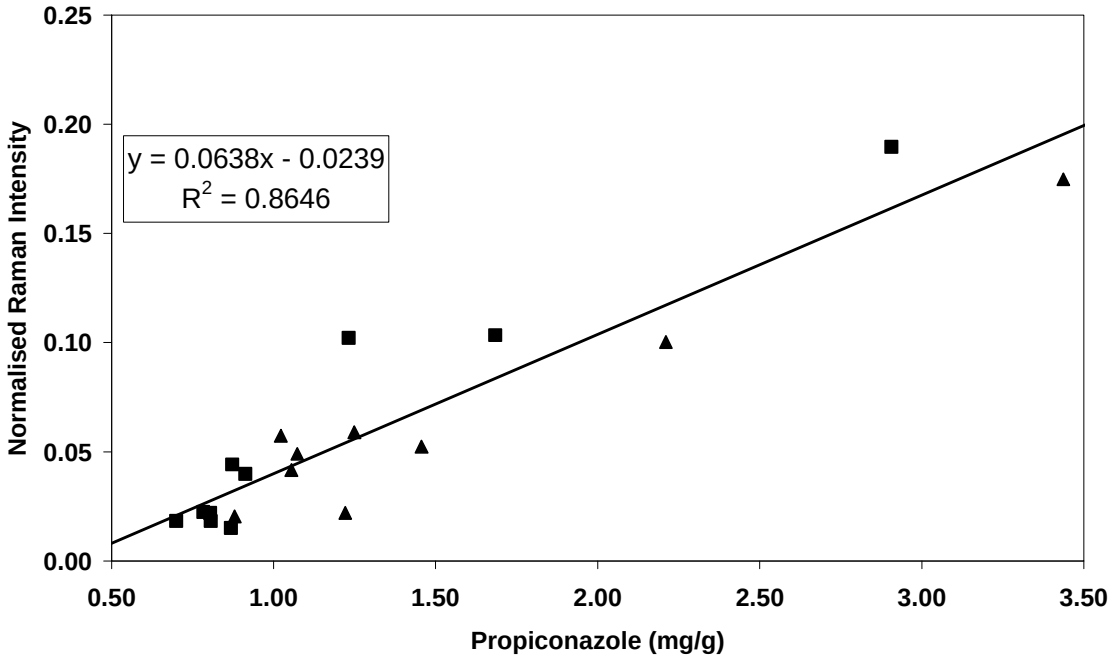

Figure 6: Calibration curve relating the average normalised Raman intensity at 647-693 cm^{-1} (propiconazole) in wood to the propiconazole concentration determined by GC-MS (data from figures 1 and 5). Squares: 48-hour sample; triangles: 96-hour sample.

4. Conclusions

Raman spectroscopy shows potential as a means of analysis of propiconazole in wood. At this point the technique lacks the precision of GC-MS; however, the advantage of speed makes the technique promising, at least for semiquantitative analysis. The major drawback is that some species of wood may not be amenable to analysis due to their high fluorescence. That problem could be overcome by using a longer wavelength laser (e.g. 1064 nm). Raman microscopy revealed that propiconazole was absent in spring wood, but present in summer wood. The inhomogeneous distribution of propiconazole in the sample leads to high scatter in the Raman data. That could be improved by using a probe that encompasses a larger area, such as a fiber-optic.

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6. Literature

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