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# The effect of *Sirex noctilio* infestation and fire damage on the chemical composition of South African-grown *Pinus patula* pulpwood

**M du Plessis[1]*, NJ le Roux[2], S Gardner-Lubbe[2], JPJ Swart[3] and T Rypstra[3]**

*[1] Mondi Ltd, PO Box 39, Pietermaritzburg 3200, South Africa*
*[2] Department of Statistics and Actuarial Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*
*[3] Department of Forest and Wood Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa*
** Corresponding author, e-mail: marius.duplessis@mondigroup.com*

The infection and association between the wood wasp *Sirex noctilio* and the fungus *Amylostereum areolatum* is responsible for large-scale tree mortality in the Midlands of the KwaZulu-Natal province in South Africa. An exploratory investigation on the effect of the infestation of trees by the wood wasp and its associated fungus on the chemical composition of *Pinus patula* pulpwood was undertaken. Various tree classes representing different levels of physiological growth stress from this infestation were compared. Together with the above stress agents, fire damage to *P. patula* trees was also considered as a possible cause of changes in pulpwood chemical composition. Chemical analyses to determine the Seifert cellulose and Klason lignin contents as well as the levels of water- and solvent-soluble extractives were conducted using published Tappi standard methods. The results indicated only negligible differences between infestation levels (tree classes) with respect to Seifert cellulose and Klason lignin contents. In contrast, highly significant differences were observed for solvent- and water-borne extractives. It is evident from the results that *P. patula* trees engage a defence strategy to counter the effects of the infestation and the resulting physiological stress. The results of the chemical analyses suggest that trees should not be harvested any younger than the intended rotation age of 12 years and at the time of harvesting all the biomass, including the infected wood, should be sent to the pulp mill. Fire-damaged trees can be utilised in the same way as healthy trees when applying the TMP process provided the charcoal on the outer bark is removed. The usefulness of a biplot to simultaneously display the various tree classes and their chemical composition is illustrated.

**Keywords:** extractive content, fire damage, Klason lignin, *Pinus patula*, Seifert cellulose, *Sirex noctilio,* thermomechanical pulping

## Introduction

The South African pulp industry is reliant on *Pinus patula* as a main constituent of the softwood raw material. However, trees in plantations can periodically be physiologically stressed due to a variety of biotic and abiotic factors. Damaged or dead trees can constitute a loss of pulpwood production to the mill, with the economic consequence of having to source the timber elsewhere on the open market.

*Sirex noctilio* (Hymenoptera: Siricidae) is recognised as a plantation pest in *Pinus* stands with significant economic consequences to the industry in the short and medium term. In 1987, 1.8 million *P. radiata* trees died in South Australia and the south-western parts of Victoria as a result of a *S. noctilio* outbreak (Haugen and Underdown, 1990). In response, an extensive inoculation program was started whereby 147 000 trees were inoculated with the parasitic nematode *Deladenus siricidicola.* Although biocontrol has been introduced in southern Africa in the form of the nematode *D. siricidicola*, introduction success, unlike in Australia, was reported to be poor (Hurley *et al.*, 2007).

In its natural range, which spans northern Africa and Eurasia (Wingfield *et al.*, 2001), *S. noctilio* is not considered a serious economic problem (Slippers *et al.*, 2001). The first sighting in South Africa of *S. noctilio* in 1994 was at Tokai near Cape Town (Baxter *et al.*, 1995; Tribe, 1995), and a new era of integrated pest management was entered after its subsequent spread to the Eastern Cape and KwaZulu-Natal. In the large monoculture stands of *P. patula* in South Africa, favourable bioclimatic conditions and general absence of natural enemies have elevated this wasp to primary pest status. A recent survey established that as much as 46% of the *P. patula* plantations in the KwaZulu-Natal Midlands area are already infested at an intensity of 6%, which can escalate to 25% unless the problem is effectively addressed (Edwards, 2006).

A mutual symbiosis exists between siricid wood wasps and *Amylostereum* spp. (Talbot, 1977; Martin, 1992). The relationship between these organisms is specialised and obligatorily species specific as far as the insect is concerned. The principal advantage for the fungus is that it is spread and inoculated into suitable wood substrates during wasp oviposition. In turn, the fungus rots and dries the wood, providing a suitable environment, nutrients and enzymes for the developing insect larvae (Slippers *et al*., 2001).

Fires usually cause variable damage to plantations and, when extensive, compartments usually are clear felled as soon as possible to control secondary damage by biotic factors and anticipated changes in the chemical composition of wood, especially extractive levels. The average fire damage to plantation areas for the 20-year period between 1985 and 2005 was 8 877 ha annually for pine species, of which 5 866 ha (66%) were seriously damaged to levels where mortality set in and hence alternative uses for the timber had to be considered (FSA, 2006).

From an economic point of view it is important to establish whether these damaging agents have a negative influence on product recovery and the quality of throughput.

In this paper, the effects of infestation by *S. noctilio* and the associated fungus (*Amylostereum areolatum*) and plantation wild fires were investigated by determining the chemical composition of pulpwood (i.e. Klason lignin, Seifert cellulose, and water- and solvent-borne extractive content) of *P. patula* trees grown in the KwaZulu-Natal Midlands in South Africa. Effects of age and infestation levels formed an integral part of this exploratory investigation. Several different classes of *P. patula* are represented in the data and this, together with the multidimensional nature of the results obtained in analysing the data, necessitated the use of canonical variate analysis (CVA) biplots for an effective graphical display of the results.

## Materials and methods

Compartments were selected from the harvesting plan followed by an in-field inspection of the various levels of *S. noctilio* infestation. Sampling was based on the degree of visible mortality and classified according to five distinct classes that also included a sample of fire-damaged trees. The procedure for sampling the tree classes infested with *S. noctilio* consisted of the selection of two representative trees from two different plantation compartments within a 5 km radius for each of the various levels of infestation, i.e. a total of 20 trees (four per class) were sampled. The five sample classes are described as follows:

- Class A: dead, 12 years old; infested by *S. noctilio* for more than two years. Typical characteristics were a dead crown, bark peeling off at places along the main stem and the timber was light weighted with only the ambient moisture levels.
- Class B: dying, 12 years old; infested by *S. noctilio* for one to two years. Typical features were needles turning brown but the logs still had significant moisture levels.
- Class C: healthy, 12 years old; at rotation end regarded as a control.
- Class D: young, healthy trees approximately eight years old.
- Class E: burnt, age unknown, logs were salvaged from a burnt compartment and kept under irrigation with water in a depot.

Burnt logs representing class E were taken from a depot at the pulp mill. Similar to the sampling for *S. noctilio* infested trees, four logs were randomly selected from the surge depot, chipped and a bulk sample selected from all the chips.

The four trees in each class were manually debarked and chipped together in a side feed Fulghum pilot chipper. The pulpwood chips were well mixed to a homogeneous blend. Chip mixtures were screened and three samples were randomly taken from the 6–12 mm fractions for chemical analysis.

The following chemical analyses were conducted on the selected material, with the reference methodology shown in brackets:

- Seifert cellulose (Browning, 1967)
- Klason lignin (TAPPI standard method number T222 om-88)
- Alcohol-benzene extractive content (TAPPI standard method number T204 om-88)
- Hot water-soluble extractive content after determining the alcohol-benzene extractive content (TAPPI standard method T264 om-88).

Canonical variate analysis biplots, regarded as extensions of ordinary scatter plots, were used to describe variation in the measured multivariate observations. Where required, 95% simultaneous confidence intervals were constructed using the Tukey method for all pairwise comparisons.

## Results

Table 1 shows the tree class means and standard deviations obtained in the chemical analyses conducted on the pulpwood. The correlation matrix associated with the four chemical components is given in Table 2. Since only a small number of samples were available for calculating the correlations in Table 2, care should be exercised when interpreting these coefficients.

The CVA biplot shown in Figure 1 is a multivariate display of the class means given in Table 1 and in which simultaneously the quantities of the four wood components are shown. Figure 1 highlights (1) the overlap of the dead, healthy and burnt trees with respect to the quantities of the four measured chemical components and (2) the complete separation of this cluster from both the dying and the young tree classes.

The biplot is ideal for visually assessing the tree class separation and overlap since the individual samples are shown about their respective class means. The four component quantities are represented by the four calibrated axes allowing for visual appraisal of the quantities responsible for differences among the tree classes. Furthermore, Figure 1 shows the individual samples to lie close to the respective tree class means, visually displaying the relatively small standard deviations given in Table 1.

Although the sample sizes were very small, an attempt was made to perform an analysis of variance (ANOVA). As a first step, a one-way multivariate analysis of variance (MANOVA) was performed. This resulted in a highly significant *p*-value leading to several univariate one-way analyses of variance. The results of these analyses are presented in Tables 3 and 4.

Confidence intervals were calculated for pairwise comparisons of the five tree classes for contents of Klason lignin, solvent-borne extractives and water-borne extractives, respectively. These intervals are given in Table 5.

## Discussion

### *Canonical variance analysis biplots*

Scatter plots and box plots, as used in Figure 1, are simple graphical devices almost universally understood and used in data analysis (Chambers *et al.*, 1983). However, in an

**Table 1:** Mean values (%) obtained in the pulpwood chemical analyses with the standard deviation in brackets. CELCON = cellulose concentration, LIGCON = lignin concentration, EXTSOL = solvent-soluble extractives concentration, $EXTH_2O$ = water-soluble extractives concentration (concentrations expressed as percentage based on oven-dry wood mass)

| Tree class | CELCON | LIGCON | EXTSOL | $EXTH_2O$ |
|---|---|---|---|---|
| A (Dead) | 43.56 (0.11) | 31.37 (1.00) | 0.93 (0.12) | 1.47 (0.23) |
| B (Dying) | 43.31 (0.97) | 32.76 (1.20) | 2.20 (0.20) | 2.07 (0.31) |
| C (Healthy) | 43.93 (1.86) | 28.54 (1.13) | 1.13 (0.12) | 1.60 (0.00) |
| D (Young) | 43.67 (1.61) | 29.18 (2.30) | 4.86 (0.12) | 1.40 (0.20) |
| E (Burnt) | 44.54 (1.04) | 31.99 (1.97) | 0.93 (0.12) | 1.07 (0.12) |

**Table 2:** Correlation matrix associated with the chemical analyses when tree class is disregarded. Abbreviations are as in Table 1

| | CELCON | LIGCON | EXTSOL | $EXTH_2O$ |
|---|---|---|---|---|
| CELCON | 1.0000 | | | |
| LIGCON | 0.3053 | 1.0000 | | |
| EXTSOL | –0.1285 | –0.2638 | 1.0000 | |
| $EXTH_2O$ | –0.4182 | 0.0471 | 0.0740 | 1.0000 |

ordinary scatter plot, only two variables (characteristics measured for each data point) can be visually displayed at a time. The biplot, introduced by Gabriel (1971), provides a means for displaying in a single graph more than two variables (i.e. characteristics measured), which was the case in this study.

Figure 1 shows a biplot, a specific two-dimensional scatter plot, showing all the sample points together on an axis for each of the chemical components determined in the wood samples. These axes are calibrated in the original units of measurement just as in an ordinary scatter plot. Although the biplot axes do not intersect at right angles they are used exactly as in an ordinary scatter plot: a line is drawn perpendicular from any point on the graph to any biplot axis and the value for that variable or property is read from the scale on the particular axis. The biplot presented in this paper is equipped with calibrated axes allowing the reading-off of values as described above. A similar approach was followed by Gardner *et al.* (2005) showing the usefulness of the biplot variant of the scatter plot as an alternative to CVA and MANOVA in the case of small sample sizes.

By visually considering Figure 1, the following can be deduced regarding the differences among the tree classes in terms of their respective chemical compositions.

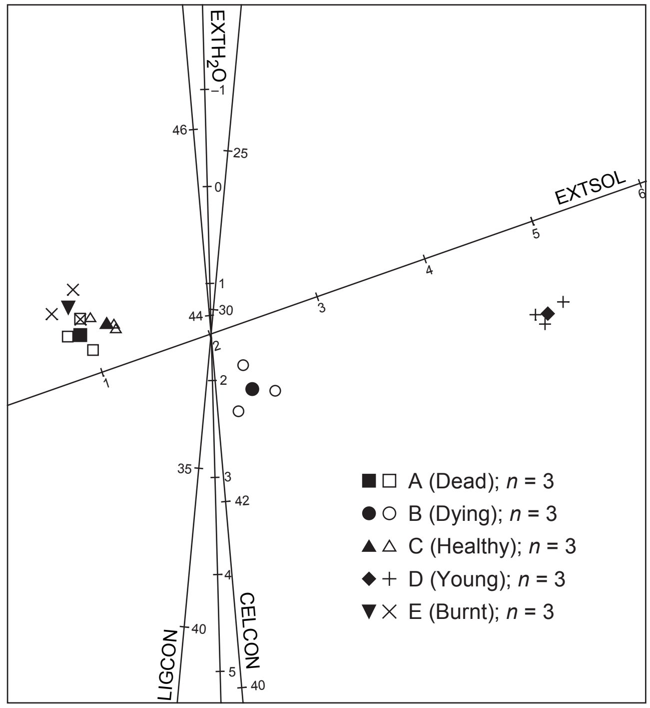


**Figure 1:** Canonical variance analysis biplot of four chemical wood components with class means (solid symbols) together with individual samples shown (hollow symbols). Abbreviations are as in Table 1

### *Extractives*

Noteworthy in Figure 1 is the homogeneity of solvent-borne extractive content means for the cluster consisting of healthy, burnt and dead trees (tree classes C, E and A), while a significantly higher solvent-borne extractive content means for the dying tree class (class B) was observed and, in turn, the dying tree class had a significantly lower mean than the young tree class (class D).

The young tree class showed the highest solvent-borne extractives mean value with the cluster of dead, healthy and burnt trees having the lowest solvent-borne extractives values. The dying tree class has a mean solvent-borne extractives value lying between the extremes of the above cluster of tree classes and the young tree class. It has been reported by Simpson and McQuilkin (1976) and Smith (2000) that monoterpenes, which are common in conifers and especially pines, are often associated with plant defence against insect attack and disease infection. Monoterpenes are extracted with alcohol-benzene. It follows from the data presented in Tables 1 and 2 that the physiological stresses associated with trees in the dying class have caused these trees to produce more solvent-borne extractives as a means of combating the effects of *A. areolatum*. The solvent-borne extractive level of 2.20% for the dying tree class is almost double the 1.13% found in the healthy tree class. The young tree class had an even higher solvent-borne extractive content of 4.86%, more than three times that of the healthy trees.

From the results it can be further conjectured that the level of solvent-borne extractives returned to a lower level

**Table 3:** Results of multivariate analysis of variance performed when the four chemical components present in the five tree classes were simultaneously used as dependent variables

| Source | df | Pillai's trace | Approximate *F* | df | *P*-value |
|---|---|---|---|---|---|
| Classes | 4 | 2.4783 | 4.0714 | 16 (numerator), 40 (denominator) | 0.00016 |
| Residuals | 10 | | | | |

**Table 4:** Results of analyses of variance performed on the five tree classes with each of Seifert cellulose, Klason lignin, solvent-borne extractives and water-borne extractives contents used in turn as the dependent variable

| Source | df | Sum of squares | Mean sum of squares | *F* | *P*-value |
|---|---|---|---|---|---|
| Dependent variable: Seifert cellulose content (%) | | | | | |
| Classes | 4 | 2.64 | 0.661 | 0.4080 | 0.7991 |
| Residuals | 10 | 16.20 | 1.620 | | |
| Dependent variable: Klason lignin content (%) | | | | | |
| Classes | 4 | 39.91 | 9.98 | 3.8747 | 0.0375 |
| Residuals | 10 | 25.75 | 2.58 | | |
| Dependent variable: solvent-borne extractives content (%) | | | | | |
| Classes | 4 | 33.80 | 8.45 | 452.2083 | 0.0000 |
| Residuals | 10 | 0.19 | 0.02 | | |
| Dependent variable: water-borne extractives content (%) | | | | | |
| Classes | 4 | 1.59 | 0.40 | 9.9194 | 0.0017 |
| Residuals | 10 | 0.40 | 0.05 | | |

once the tree died. Conspicuous in the case of the dead tree class were resin droplets exuded on the stem surface, visible on the outer bark.

In the case of water-borne extractives content, Table 5 shows that the water-borne extractives content means of the dying tree class (class B) was statistically higher than for dead, young or burnt tree classes (classes A, D and E). Not only are all these differences and similarities visually displayed in Figure 1, but the biplot takes into account the multidimensional nature of the data.

### *Lignin*

The dying tree class had the highest Klason lignin and solvent-borne extractives means and the lowest Seifert cellulose mean. As the dying of infected trees is a physiological process over time, it must be borne in mind that results shown here are from samples taken at various stages of the dying process and from different trees and localities. It follows from Table 5 that no pairwise difference between any two Klason lignin contents means could be considered statistically significant.

### *Cellulose*

Table 4 indicates that there was no significant difference among the mean Seifert cellulose contents of the tree classes but that the means of the other chemical components differed significantly. It was also concluded from studies by Werner *et al.* (1983) and Zhu and Myers (2006) that the cellulosic pulp yield for dead trees infected by insects and blue stain fungus was not different from that of live trees. Similar results were obtained by Scott *et al.* (1997) showing that beetle-killed white spruce (*Picea glauca)* wood pulped equally as well as unaffected wood.

## Conclusion

We have demonstrated the capabilities of a CVA biplot when regarded as a multivariate extension of an ordinary scatter plot for providing information regarding overlap and separation of *P. patula* trees infected by *S. noctilio* and categorised in five classes for four main chemical compounds found in wood.

In this exploratory investigation, the Seifert cellulose content of the five tree classes showed only negligible differences. Seifert cellulose content was thus not adversely affected by either infestation of *S. noctilio* and its associated fungus *A. areolatum,* or fire damage.

The ANOVA conducted on Klason lignin content showed there may be differences between tree classes but this was not substantiated by a pairwise comparison procedure.

For the solvent-borne extractives content, a cluster consisting of healthy, burnt and dead trees was formed exhibiting a relatively low solvent-borne extractive value. This cluster differed significantly from both the dying and the young trees, which in turn also differed significantly from one another.

The dying tree class exhibited the highest water-borne extractives level, whereas the relative level of solvent-borne extractives of the young tree class was exceptionally high. It is also clear that the defence mechanism of *P. patula* trees subjected to physiological stresses caused by *S. noctilio* was in dying trees manifested in increased levels of resin production. The finding that the solvent-borne extractives level of dying trees was significantly higher than the corresponding level for healthy trees, approaching that of the young trees, and in the case of dead trees dropped again to that of the healthy trees, can be hypothesised as the tree's response to fight external influences that are threatening its existence.

**Table 5:** 95% Simultaneous confidence intervals for the pairwise differences for mean percentage contents of lignin, solvent-borne extractives and water-borne extractives

| Difference | Estimate | SE | Lower bound | Upper bound | Excluding zero |
|---|---|---|---|---|---|
| Mean Klason lignin content (%) | | | | | |
| A–B | –1.390 | 1.31 | –5.700 | 2.920 | No |
| A–C | 2.830 | 1.31 | –1.490 | 7.140 | No |
| A–D | 2.190 | 1.31 | –2.120 | 6.500 | No |
| A–E | –0.625 | 1.31 | –4.940 | 3.690 | No |
| B–C | 4.210 | 1.31 | –0.097 | 8.530 | No |
| B–D | 3.580 | 1.31 | –0.732 | 7.890 | No |
| B–E | 0.764 | 1.31 | –3.550 | 5.080 | No |
| C–D | –0.635 | 1.31 | –4.950 | 3.680 | No |
| C–E | –0.450 | 1.31 | –7.760 | 0.861 | No |
| D–E | –0.820 | 1.31 | –7.130 | 1.500 | No |
| Mean solvent-borne extractives content (%) | | | | | |
| A–B | –1.2700 | 0.112 | –1.640 | –0.901 | Yes |
| A–C | –0.1990 | 0.112 | –0.567 | 0.168 | No |
| A–D | –3.9300 | 0.112 | –4.300 | –3.560 | Yes |
| A–E | 0.0001 | 0.112 | –0.367 | 0.367 | No |
| B–C | 1.0700 | 0.112 | 0.701 | 1.440 | Yes |
| B–D | –2.6600 | 0.112 | –3.030 | –2.290 | Yes |
| B–E | 1.2700 | 0.112 | 0.901 | 1.640 | Yes |
| C–D | –3.7300 | 0.112 | –4.100 | –3.360 | Yes |
| C–E | 0.1990 | 0.112 | –0.168 | 0.567 | No |
| D–E | 3.9300 | 0.112 | 3.560 | 4.300 | Yes |
| Mean water-borne extractives content (%) | | | | | |
| A–B | –0.6010 | 0.163 | –1.14000 | –0.0632 | Yes |
| A–C | –0.1320 | 0.163 | –0.67000 | 0.4050 | No |
| A–D | 0.0685 | 0.163 | –0.46900 | 0.6060 | No |
| A–E | 0.4000 | 0.163 | –0.13700 | 0.9380 | No |
| B–C | 0.4690 | 0.163 | –0.06900 | 1.0100 | No |
| B–D | 0.6690 | 0.163 | 0.13200 | 1.2100 | Yes |
| B–E | 1.0000 | 0.163 | 0.46400 | 1.5400 | Yes |
| C–D | 0.2010 | 0.163 | –0.33700 | 0.7380 | No |
| C–E | 0.5330 | 0.163 | –0.00509 | 1.0700 | No |
| D–E | 0.3320 | 0.163 | –0.20600 | 0.8700 | No |

Based on the chemical properties of the wood, the above discussion suggests the following. Trees infested with *Sirex noctilio* should not be harvested any younger than the intended rotation age (12 years) and at the time of harvesting all the biomass, including the infected wood, should be sent to the pulp mill. This will ensure minimum solvent-borne extractives levels (resin content) and improved production of thermo-mechanical pulps. Moreover, as is clearly seen in the biplot in Figure 1**,** the dead tree class compared favourably with the healthy tree class in terms of Seifert cellulose, Klason lignin contents and water-borne extractives levels.

Determination of the levels of solvent-borne extractives can serve as a potential method to determine the degree of physiological stress that a tree is subjected to. Higher resin levels indicate the tree's degree of reaction to damage.

Trees infested with *Sirex noctilio* and fire-damaged trees can be utilised in the same way as healthy trees when using the TMP process.

The following question arises from these conclusions: if on the chemical level cellulose content of infested trees is not affected while other chemical properties are affected, are pulp yield and associated properties such as energy consumption, brightness, burst strength, tear strength, tensile strength and breaking length also affected? TMP pulp and paper tests are currently in progress to investigate these aspects of tree response to *S. noctilio* infestation.

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