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Variation in foliar water content and hyperspectral reflectance of *Pinus patula* trees infested by *Sirex noctilio*

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The remote detection and quantification of symptoms associated with declining forest health is critical for the introduction of proper pest monitoring and control measures. *Sirex noctilio,* the Eurasian wood wasp, is one of the major pests responsible for declining forest health in pine forests located in KwaZulu-Natal, South Africa. Researchers have shown that stress induced by *S. noctilio* causes a rapid decrease in foliar water content, with the foliage of the tree changing from a dark green to a reddish brown hue. This study examined if the variation in foliar water content due to *S. noctilio* infestation can be remotely detected. Foliar water content and *in situ* hyperspectral measurements were obtained from *Pinus patula* trees experiencing varying levels of stress induced by *S. noctilio*. Subsequently, foliar water content was correlated to selected spectral variables consisting of known water absorption features, spectral indices and continuum-removed absorption features. Results showed that the variations in foliar water content across the varying levels of *S. noctilio* infestation were strongly linked to the variation in hyperspectral reflectance. Except for water absorption features located at 970 nm and 1200 nm, there was a strong correlation between the majority of the spectral variables and foliar water content. Of the spectral variables tested, the water index (WI) provided the strongest linear correlation ($r = 0.84$) with foliar water content. Ultimately, results obtained from this study provide the foundation for the detection and monitoring of *S. noctilio* infestations at a landscape level using airborne or spaceborne hyperspectral platforms.

Keywords: absorption features, continuum-removed absorption features, foliar water content, hyperspectral, *Sirex noctilio*, spectral indices

Introduction

The remote detection, identification and quantification of symptoms associated with declining forest health is crucial for forest managers and scientists who are interested in monitoring and managing forest health at local and global scales. The Eurasian wood wasp, *Sirex noctilio*, is the primary pest responsible for the systematic decline of forest health in commercial pine forests located in South Africa (Hurley et al. 2007, Ismail et al. 2007, Slippers 2006). In the province of KwaZulu-Natal, the wasp predominately attacks mature *Pinus patula* trees (Hurley et al. 2007). Unabated, the wasp population may develop into epidemic proportions, resulting in widespread *P. patula* mortality at a landscape level (Slippers 2006).

The physiological effects of *Pinus* trees experiencing *S. noctilio* induced stress has been a subject of investigation for several years (Coutts 1965, 1968, 1970, Slippers et al. 2003). Research has shown that after an initial flight period, the female wasp inserts her ovipositor through the bark into the sapwood and lays up to three eggs at each drill site (Madden 1974, Spradberry 1977). During the process, the wasp also introduces a toxic mucus secretion and the arthrospores of the symbiotic fungus *Amylostereum areolatum* into the tree (Slippers et al. 2003). The mucus secreted by the wasp changes the water balance of the tree thereby creating conditions that are ideal for the growth and spread of the fungus (Slippers et al. 2003). More specifically, Coutts (1970) observed that trees injected only with the mucus secretions do not die. The mucus changes the physiological conditions of the tree, resulting in an accumulation of phosphate and an increase in the dry weight of the foliage. However, trees that were injected with the mucus and the fungus showed a rapid decrease in foliar moisture content within 2–3 weeks, resulting in the tree dying with the foliage changing from a dark green to a reddish brown hue.

In an effort to develop a pest monitoring framework, the visual symptoms associated with *S. noctilio* infestations were divided into a damage scale by Ismail et al. (2007). The damage scale progresses from healthy (no indication of attack) to green (appearance of resin droplets and presence of ovipositors), and finally to red (wilting of the attacked tree and leaves appearing reddish brown). Based on the physiological observations of Coutts (1970) and the damage scale of Ismail et al. (2007), we hypothesise that the foliage of the infested trees will have variations in foliar water content depending on the scale of damage. If that holds, then the remote detection and mapping of the foliar water content of *P. patula* infested by *S. noctilio* would help in accurately quantifying the severity of damage caused by the wasp.

Developments in hyperspectral remote sensing have shown that spectral reflectance curves vary with the amount of foliar water content present in plants (Gausman et al. 1971, Inoue et al. 1993, Pu et al. 2003, Stimson et al. 2005). Subsequently, several spectral indices and specific water absorption features have been used to estimate foliar water content. For example, research carried out by Cohen (1991a, 1991b) showed that ratios of the red to near infrared (NIR), and the NIR to the shortwave infrared (SWIR), were correlated with the water status of *Pinus coulteri* and *P. contorta* needles. Stimson et al. (2005) used spectral indices such as the normalised difference vegetation index (NDVI), the normalised difference water index (NDWI), the red edge, and continuum-removed absorption features (located at 970 nm and 1 200 nm) to detect foliar drought stress present in *Pinus edulis* and *Juniperus monosperma*. Their results showed that a strong link exists between plant physiological characteristics related to water stress and spectral reflectance. Regarding other forest genera, Pu et al. (2003) investigated water stress in *Quercus agrifolia* as a result of sudden oak disease and found that a linear relationship exists between the foliar water content and spectral variables derived from the water absorption bands located at 975 nm, 1 200 nm, and 1 750 nm. More recently, Eitel et al. (2006) examined the ability of several spectral indices to remotely detect water stress in *Populus* species. Results from the study indicated that statistically significant but poor relationships were obtained at a leaf level using spectral indices such as NDVI, water index (WI) and the red edge.

However, in spite of the wide use of water absorption bands, spectral indices and continuum-removed features, researchers have not established whether there are any variations in the quantity of foliar water and spectral reflectance for the healthy, green and red stages of *S. noctilio* infestation. In an effort to improve current detection and monitoring methods, this study aimed to search for spectral variables that will hopefully detect the variation in foliar water content of *P. patula* trees that are experiencing varying levels of stress induced by *S. noctilio*.

Materials and methods

Field data collection

Pinus patula foliage was collected from a *S. noctilio* infested compartment located at the Sappi Pinewoods plantation (centroid 29°38′36.06″ S, 30°4′13.83″ E) in KwaZulu-Natal, South Africa (Ismail et al. 2008). To facilitate a representative sample, *P. patula* trees were carefully examined with the assistance of experienced foresters and classified into mutually exclusive classes (i.e. healthy, green and red). Using tree climbers, samples representing each class (healthy = 24, green = 30 and red = 12) were randomly obtained from the upper, middle and lower crowns of selected trees (Ismail et al. 2008). After clipping, each sample (approximately 1 kg) was immediately placed on the ground and spectral measurements were collected. The measurements were taken on a clear sunny day between 10:00 and 14:00, using an Analytical Spectral Devices Field Spec Pro FR spectroradiometer. The spectroradiometer senses in the 350–2 500 nm spectral range. The first sensor measures reflection in wavelengths between 350 nm and 1 050 nm with a spectral resolution of 1.4 nm, while the second sensor measures reflection between 1 000 nm and 2 500 nm with a spectral resolution of 2 nm (Analytical Spectral Devices 2002). In accordance with established protocols, the spectroradiometer was mounted on a tripod with a 25° field of view and positioned 0.5 m above each sample at nadir position. Additionally, radiance measurements were converted to target reflectance using a calibrated white spectralon panel of known spectral characteristics (Analytical Spectral Devices 2002). To control for variation in leaf orientation, 10 spectral reflectance measurements were averaged for each sample and individual samples were rotated 30° between scans (Pontius et al. 2005).

Water content analysis

Following the spectral measurements, foliar samples from each class (i.e. healthy, green and red) were immediately sealed in a plastic bag and sent to the Institute of Commercial Forestry Research laboratory, Pietermaritzburg, South Africa, for water content analysis. Foliar samples were weighed fresh (fresh weight; FW) and then dried in an oven for approximately 24 h at 60 °C. The leaf samples were then weighed again after drying (dry weight; DW). Foliar water content (WC) was calculated as the ratio between the quantity of water (FW − DW) and the FW (Ceccato et al. 2001, Bowyer and Danson 2004):

$$\mathrm{WC}\ (\%) = ((\mathrm{FW} - \mathrm{DW}) / \mathrm{FW}) \times 100 \quad (1)$$

Spectral variables

In addition to using known water absorption features located near or at 970 nm, 1 200 nm and 1 400 nm (Curran 1989, Pu et al. 2003), several spectral variables that included spectral indices and continuum-removed features were also computed from the hyperspectral dataset.

Spectral indices were selected due to their previous success in detecting water stress (Pu et al. 2003, Stimson et al. 2005, Eitel et al. 2006) and calculated as a ratio between wavelengths located from the water absorption region of the electromagnetic spectrum and a control wavelength located outside the water absorption region (Sims and Gamon 2003). More specifically, the spectral indices considered in this study included the water index (Penuelas et al. 1997), the normalised difference water index (Hardinsky et al. 1983), the normalised difference vegetation index (Rouse et al. 1973) and three-band ratio indices (Gao et al. 1993, Pu et al. 2003).

The water index (WI) is based on the ratio between the reflectance (R) located at 900 nm and at 970 nm (Penuelas et al. 1997), and is calculated as:

$$\mathrm{WI} = R_{900} / R_{970} \quad (2)$$

The normalised difference water index (NDWI) ratios the reflectance at 860 nm and 1 240 nm (Hardinsky et al. 1983), and is calculated as:

$$\mathrm{NDWI} = (R_{860} - R_{1240}) / (R_{860} + R_{1240}) \quad (3)$$

The normalised difference vegetation index (NDVI) is calculated using reflectance values located at 860 nm and 690 nm (Rouse et al. 1973) as follows:

$$\mathrm{NDVI} = (R_{860} - R_{690}) / (R_{860} + R_{690}) \quad (4)$$

The three-band ratio indices (Gao et al. 1993, Pu et al. 2003) for the absorption wavelengths centered at 975 nm and 1 200 nm were calculated as follows:

$$\mathrm{Ratio}_{975} = 2R_{960–990} / (R_{920–940} + R_{1090–1110}) \quad (5)$$

$$\mathrm{Ratio}_{1200} = 2R_{1180–1220} / (R_{1090–1110} + R_{1265–1285}) \quad (6)$$

Continuum removal was applied to the water absorption features in an effort to reduce background effects and normalise the spectral reflectance of these features (Kokaly and Clark 1999). The continuum is calculated by fitting a convex hull over the top of the spectrum, utilising straight line segments that connect local spectra maxima (Clark and Roush 1984, Kokaly and Clark 1999). The continuum is then removed by dividing the reflectance value for each point in the absorption features by the reflectance level of the continuum line (convex hull) at the corresponding wavelength (Mutanga et al. 2003, Huanga et al. 2004). Removing the continuum thus standardises isolated absorption features for comparison purposes (Clark 1999). Figure 1a shows the spectral reflectance fitted with a convex hull and Figure 1b shows the continuum-removed reflectance that was derived from Figure 1a.

Continuum removal was applied to water absorption features located at $R_{920–1120}$ and $R_{1070–1320}$ (Pu et al. 2003). Although several variables can be calculated from these continuum-removed absorption features (Clark and Roush 1984, Kokaly and Clark 1999, Mutanga et al. 2003, Pu et al. 2003), we only calculated band depth (BD), which is computationally efficient and therefore more suitable for the practical application of this study (Mutanga and Skidmore 2004). Band depth at 975 nm (BD_{975}) and band depth at 1 200 nm (BD_{1200}) were calculated by subtracting the continuum-removed reflectance R' at wavelength i from 1:

$$\mathrm{BD}_{(\lambda i)} = 1 - R'_{(\lambda i)} \quad (7)$$

where i represents the 970 nm and 1 200 nm wavelengths.

Results

Variation in water content

The average water content was 58.93% for the healthy class, 45.60% for the green class and 14.67% for the red class. The range of foliar water content varied from a maximum of 63.55% for the healthy class to a minimum of only 9.83% for the red class (Table 1).

We used a one-way analysis of variance (ANOVA) to test whether the differences in foliar water content between the different classes were significant. We tested the research hypothesis that the mean foliar water content (%) measured from the healthy, green and red classes were different, viz. the null hypothesis H_0: $\mu_1 = \mu_2 = \mu_3$ versus the alternate hypothesis H_a: $\mu_1 \neq \mu_2 \neq \mu_3$, where μ_1, μ_2 and μ_3 are the mean foliar water content for the healthy, green and red classes. Results from the ANOVA showed that the mean foliar water content for the three classes differed significantly (F value = 170.83, $p < 0.001$).

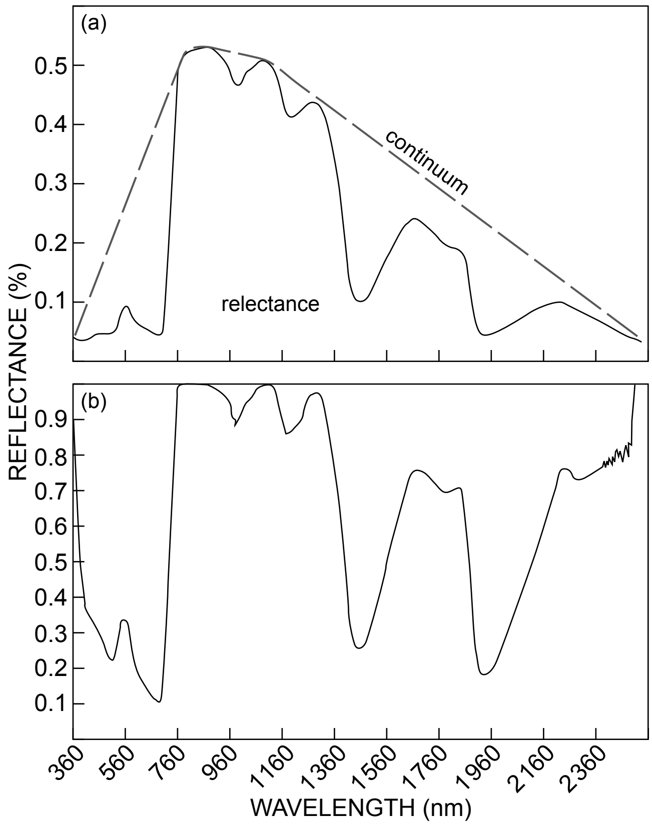

Figure 1: (a) The laboratory spectral reflectance of healthy *P. patula* needles fitted with a convex hull and (b) the continuum-removed reflectance that was derived from (a)

Table 1: Variation in foliar water content (%) for the red (n = 12), green (n = 24), and healthy (n = 30) classes

Summary statistics	Red	Green	Healthy
Minimum	9.83	25.70	51.54
Maximum	22.96	62.73	63.55
Average	14.67	45.60	58.93
Standard deviation	5.55	12.33	11.84

Subsequently, we ran a *post hoc* Tukey HSD test in order to establish whether there were any significant differences in foliar water content between each class pair (i.e. healthy-red, healthy-green and green-red). The test was done to complement the results obtained from the ANOVA, which showed that there was a significant difference in the mean foliar water content for all three classes, but did not show which pairs were different. Results from the *post hoc* Tukey HSD test indicated that the mean foliar water content differed significantly between the healthy-red, healthy-green and green-red class pairs ($p < 0.001$).

Variation in wavelength reflectance

Figure 2 shows that there is a clear visual difference in reflectance between the three classes of *S. noctilio* infestation. In the visible part of the electromagnetic spectrum (350–700 nm) the red and green classes had a high reflectance in the red region (600–700 nm) when compared to the healthy class. In contrast, there was a decrease in reflectance in the NIR region (700–1 300 nm) for the green and red classes. In the SWIR (1 300–2 500 nm), the red class has highest reflectance followed by the green class. A more detailed description and explanation of the variation in spectral reflectance of individual bands due to *S. noctilio* infestation is provided by Ismail et al. (2008). In this study we will specifically focus on the water absorption features centred near 970 nm, 1 200 nm and 1 450 nm (Figure 2), because these bands are known to be affected by variation in foliar water content (Curran 1989, Pu et al. 2003).

Figure 3 shows the distribution and mean reflectance values for the red, green and healthy classes for the water absorption bands located at 970 nm, 1 200 nm and 1 450 nm. For the water absorption bands located in the NIR (970 nm and 1 200 nm) the mean reflectance for the healthy class was higher than the other classes. Conversely, for the water absorption band located at 1 450 nm, the mean reflectance values for the healthy class was much lower than the other classes of infestation. Results obtained from the ANOVA with a *post hoc* Tukey HSD test confirmed that the mean reflectance for the water absorption bands used in this study were significantly different ($p < 0.001$) between all three classes for the water absorption bands located at 970 nm, 1 200 nm, and 1 450 nm. Based on these results we expected the spectral variables (i.e. spectral indices and continuum-removed absorption features) at these water absorption bands to detect the variation of water content as a result of *S. noctilio* infestation.

Relationship between water content and spectral variables

We ran a correlation analysis between foliar water content and the spectral variables (i.e. water absorption bands, spectral indices and continuum-removed features). Table 2 shows the results of the correlation analysis. Except for the wavelengths located at 970 nm ($r = 0.26$) and 1 200 nm ($r = 0.19$), there was a strong significant correlation between the spectral variables used in this study and water content ($p < 0.001$).

While the correlations between the reflectance values of bands located at 970 nm and 1200 nm and foliar water content were weak, the three-band ratio indices and continuum-removed features that use these bands yielded a high correlation. For example, BD_{975} and BD_{1200} have positive correlation values of 0.71 and 0.79, whereas $Ratio_{975}$ and $Ratio_{1200}$ have negative correlation values of 0.81 and 0.76. Overall, the water index (WI) yielded the strongest linear correlation ($r = 0.84$, $p < 0.001$) with foliar water content. Figure 4 shows the distribution of WI values for the red, green and healthy classes while Figure 5 shows the linear relationship between WI and foliar water content. Noticeable in Figure 4 is the high WI values for the healthy class when compared to the low WI values of the red class.

In order to examine the robustness and sensitivity of the spectral variables used in this study, the red-stage samples ($n = 12$) were removed from the correlation analysis.

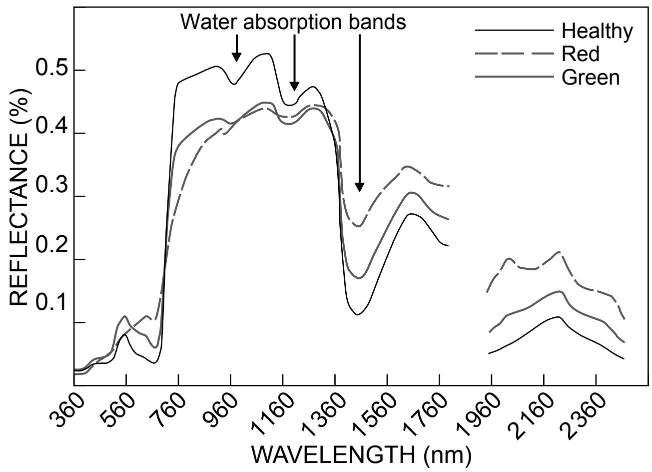

Figure 2: Spectral reflectance variation for the healthy, green and red stages of *Sirex noctilio* infestation. Reflectance values between 1800 nm and 1950 nm, and between 2470 nm and 2500 nm, displayed a high level of noise and were therefore removed from further analysis

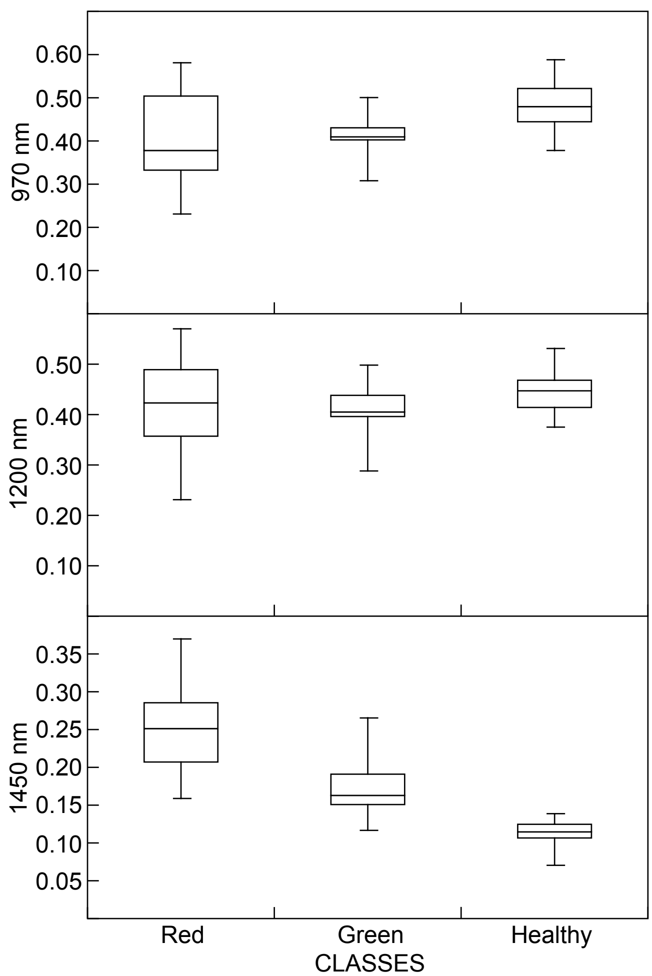

Figure 3: Box plots showing the distribution and mean reflectance values for the red, green and healthy classes for the water absorption bands located at 970 nm, 1200 nm and 1450 nm

Table 2: Relationship between foliar water content ($n = 66$) and spectral variables

	Spectral feature	Wavelength/ index	Correlation coefficient
1	Water absorption wavelength	970 nm	0.26
2	Water absorption wavelength	1200 nm	0.19
3	Water absorption wavelength	1450 nm	−0.75**
4	Spectral index	NDVI	0.77**
5	Spectral index	WI	0.84**
6	Spectral index	NDWI	0.73**
7	Spectral index	$Ratio_{975}$	−0.81**
8	Spectral index	$Ratio_{1200}$	−0.76**
9	Continuum removed	BD_{975}	0.71**
10	Continuum removed	BD_{1200}	0.79**

** Significant ($p < 0.001$)

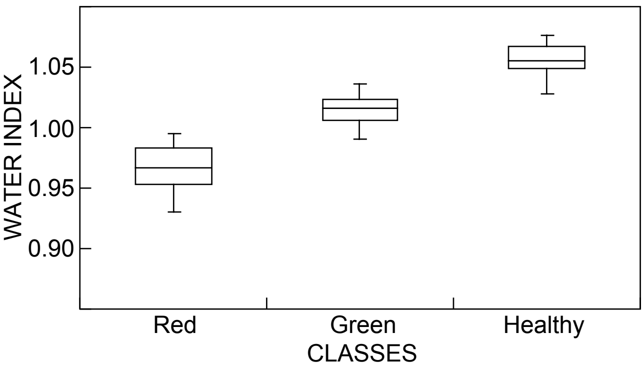

Figure 4: Box plot showing the distribution and mean reflectance of the water index (WI) values for the red, green and healthy classes

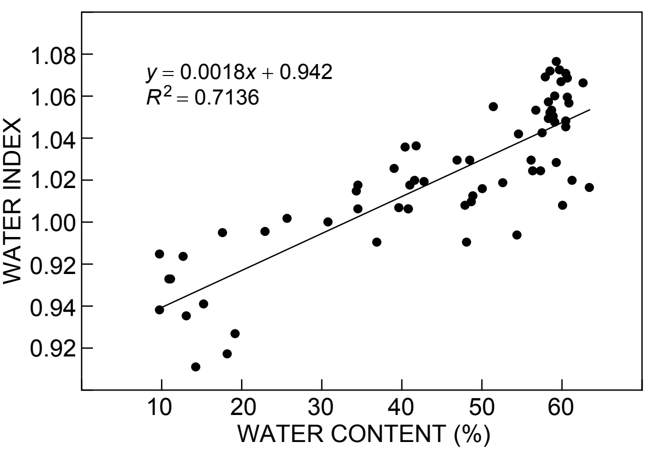

Figure 5: Linear regression model of water content (%) and the water index (WI)

Additionally, we suspected that samples from the red class were amplifying the correlation coefficients of the spectral variables due to their low water content values (minimum of 9.83% and a maximum of 22.96%). Table 3 shows the results of the correlation analysis when the red class was excluded from the study. Overall, there was a decrease in the correlation coefficients for all the spectral variables considered in the study when the red class was excluded from the analysis. The $Ratio_{975}$ spectral index yielded the highest decrease in correlation (19%) followed by reflectance at 1 450 nm (17%), WI (17%), and BD_{1200} (17%). However, in comparison to the other spectral variables examined in this study, WI, again, yielded the strongest linear correlation ($r = 0.67$, $p < 0.001$) with foliar water content.

Table 3: Relationship between the foliar water content (%) and the spectral variables used in the study. The red class (n =12) was excluded from the correlation analysis

Wavelength/index	Correlation coefficient
1450 nm	−0.58
NDVI	0.65
WI	0.67
NDWI	0.57
$Ratio_{975}$	−0.62
$Ratio_{1200}$	−0.63
BD_{975}	0.63
BD_{1200}	0.62

Discussion

Quantifying the effects of *S. noctilio* infestation on the foliar water status of *P. patula* trees will improve our ability to remotely detect and map the damage caused by the wasp. In this regard, techniques based on hyperspectral remote sensing provide an ideal platform for quantifying the variation in foliar water status associated with *S. noctilio* infestation. Experimental results from this study show that there is a significant variation in foliar water content across all three stages of *S. noctilio* infestation, increasing from a mean of 9.83% for red-stage trees to 63.55% for the healthy trees. Additionally, results indicate that there are statistically significant differences in foliar water content amongst the three classes of *S. noctilio* infestation considered in this study. Thus, the results tie up with the physiological explanation that the mucus and the fungus injected by the wasp cause a rapid decrease in the moisture content of the tree (Coutts 1970) .

With regard to the variation in foliar water content and spectral reflectance, wavelengths located at 970 nm and 1 200 nm (NIR) yielded poor correlations with foliar water content compared to the wavelength located at 1 450 nm (SWIR). These results are consistent with previous studies which have indicated that wavelengths located in the SWIR are more sensitive to changes in plant water status than wavelengths located in the NIR (Tucker 1980, Carter 1991, Eitel et al. 2006). However, it is interesting that the continuum-removed features (BD_{975} and BD_{1200}) and the spectral indices ($Ratio_{975}$ and $Ratio_{1200}$) calculated from these poorly correlated wavelengths located at 970 nm ($r = 0.26$) and 1 200 nm ($r = 0.19$) yielded strong positive correlation with foliar water content ($r > 0.7$, $p < 0.001$). These results support the notion that (1) continuum removal normalises and enhances absorption features thereby yielding a strong relationship with the variable under investigation (Kokaly and Clark 1999, Mutanga et al. 2003, Stimson et al. 2005) and (2) spectral indices will perform better than their individual wavelengths due to their ability to minimise the spectral variability caused by the morphology and biochemical properties of the foliage (Curran 1989), thereby optimising the sensitivity of the spectral reflectance of plants to changes in foliar water content (Eitel et al. 2006).

Overall, the results of this study confirm previous research that showed that variations in foliar water content due to stress may be estimated using spectral variables (Pu et al. 2003, Stimson et al. 2005, Eitel et al. 2006). The water index (WI), which is based on a ratio of two bands (900 nm and 970 nm) in the NIR, produces the best results ($r = 0.84$) for detecting the variation in foliar water content. Similar results were obtained by Dawson et al. (1999) and Bowyer and Danson (2004) who showed that WI obtained strong correlations with foliar water content. However, upon further investigation, results elucidated that the correlations of foliar water content to WI were amplified by the red class, which had significantly lower foliar water content values (greater dry weight) than the other classes. According to Bowyer and Danson (2004), stronger correlations between foliar water content (as determined by equation 1) and WI are obtained when there are low water content values (i.e. including the red class) and weaker correlations are obtained when there are higher water content values (i.e. excluding the red class) because WI is primarily affected by variations in leaf weight (i.e. dry weight) rather than by changes in foliar water content. However, compared to other spectral variables examined in this study WI still produced better correlations and shows promise in discriminating the green class or the early stages of *S. noctilio* infestations, a task that was not possible using multispectral sensors (Ismail et al. 2006).

In summary, the results from this study show that a number of spectral variables derived from hyperspectral reflectance can be used to assay the water content of *P. patula* needles experiencing stress induced by *S. noctilio*. Furthermore, the results from this study provide the impetus for the remote detection and mapping of *S. noctilio* at a landscape level. Although the relationship obtained at a needle level is insufficient to allow detection and mapping at a landscape level, it establishes a foundation for the potential upscaling of results to either an airborne or spaceborne platform (Eitel et al. 2006). For verification purposes a detailed study that examines the detection of stress induced by *S. noctilio* at a canopy level is needed. Upscaling the results of this study to a canopy level is pertinent since it is envisaged that the South African space agency will soon launch the ZASat-003 satellite (Scholes and Annamalai 2006, Mutanga et al. 2009). ZASat-003 will carry a full multisensor microsatellite imager (MSMI) instrument as well as a hyperspectral sensor that will slice the spectrum between 400 nm and 2 350 nm into 200 bands (Mutanga et al. 2009) thus providing the necessary spectral resolution needed to detect and map *S. noctilio* infestations at a landscape level.

Conclusion

This study has shown that the variation in foliar water content across the three levels of *S. noctilio* infestation in pine plantation forests is strongly linked to a variation in spectral reflectance. Additionally, the results demonstrate a critical link between the physiological effects of *S. noctilio* infestation and spectral variables derived from hyperspectral data, thus providing the foundation for the detection and monitoring of *S. noctilio* infestations at a landscape level using airborne or spaceborne hyperspectral platforms.

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