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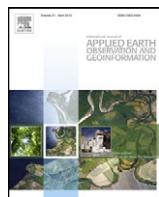
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Hyperspectral reflectance of leaves and flowers of an outbreak species discriminates season and successional stage of vegetation

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ABSTRACT

Spectral reflectance can be used to assess large-scale performances of plants in the field based on plant nutrient balance as well as composition of defence compounds. However, plant chemical composition is known to vary with season – due to its phenology – and it may even depend on the succession stage of its habitat. Here we investigate (i) how spectral reflectance could be used to discriminate successional and phenological stages of *Jacobaea vulgaris* in both leaf and flower organs and (ii) if chemical content estimation by reflectance is flower or leaf dependent.

We used *J. vulgaris*, which is a natural outbreak plant species on abandoned arable fields in north-western Europe and studied this species in a chronosequence representing successional development during time since abandonment. The chemical content and reflectance between 400 and 2500 nm wavelengths of flowers and leaves were measured throughout the season in fields of different successional ages. The data were analyzed with multivariate statistics for temporal discrimination and estimation of chemical contents in both leaf and flower organs.

Two main effects were revealed by spectral reflectance measurements: (i) both flower and leaf spectra show successional and seasonal changes, but the pattern is complex and organ specific (ii) flower head pyrrolizidine alkaloids, which are involved in plant defence against herbivores, can be detected through hyperspectral reflectance. We conclude that spectral reflectance of both leaves and flowers can provide information on plant performance during season and successional stages. As a result, remote sensing studies of plant performance in complex field situations will benefit from considering hyperspectral reflectance of different plant organs. This approach may enable more detailed studies on the link between spectral information and plant defence dynamics both aboveground and belowground.

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1. Introduction

Spatial and temporal variation in plant chemical properties results from exposure to biotic and abiotic factors in the environment. To study how plant interactions with the environment result in vegetation patterns, ecological research develops hypotheses based on observations in temporal or spatial transects such as occurring in field chronosequences. These hypotheses are tested by experimental manipulations of plants under controlled conditions in fields, greenhouses or in the laboratory (Clements, 1963; Kardol et al., 2006; van Dam et al., 1993). However, studies on changed species interactions through time are often laborious and difficult to carry out on a large spatial scale. Visible and near infra-red (VNIR)

spectroscopy offers the opportunity to study the biochemical and phenological state of plants and investigate how spectral input can aid the understanding of plant temporal processes that vary with ecosystem succession (Liu et al., 2008; Knox et al., 2010; Zeng et al., 2010; Rautiainen et al., 2009).

Hyperspectral sensors provide comprehensive spectral information of plants that allow for e.g. identifying species (Mutanga et al., 2003; Asner and Martin, 2011; Martin et al., 1998; Schmidt and Skidmore, 2001; Buddenbaum et al., 2005), analyses of plant chemical content (Knox et al., 2010; Ramoelo et al., 2011a; Card et al., 1988; Wessman et al., 1988; Fourty and Baret, 1998; Curran et al., 1992) and soil nutrient impact on a plant's (Asner and Martin, 2011; Pretorius et al., 2011). So far, few studies have addressed temporal spectral variation as a result of species responses to stages of vegetation succession. Although many temporal studies have discerned seasonal impacts on issues such as vegetation quality for food for mammals (Knox et al., 2010; Zeng et al., 2008, 2010; Gilmore et al., 2008; Hall-Beyer, 2003; Lechowicz and Koike, 1995;

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Mutanga et al., 2004; Cartelat et al., 2005; Skidmore et al., 2010), the lack of such succession-seasonal studies in other trophic levels, such as insects and soil herbivores, is certainly constrained by elegant detection methods that reveal the nature and strength of these biotic stressors in the field. Nevertheless soil microorganisms and insects are drivers of ecosystem changes (de Beurs and Townsend, 2008) and using hyperspectral reflectance could be an interesting avenue to study this more concealed biotic effects on plant performance (Carvalho et al., 2012). One such site currently available is a chronosequence of abandoned, ex-arable fields in the Netherlands, that has been studied and monitored in order to study the role of soil biota in secondary vegetation succession (van de Voorde et al., 2012).

Numerous studies have been undertaken to understand the ecological processes driving vegetation succession (Walker and del Moral, 2003). More recently, the role of soil biota (such as fungi, bacteria and invertebrate root herbivores) and their impact on plant quality and community composition have been taken into account (Kardol et al., 2006; Bezemer et al., 2006; Van de Voorde, in press). *Jacobaea vulgaris* has been a model species for many of these ecological studies, amongst others because of its hepatotoxic characteristics towards humans, cattle and invertebrates. It is known that *J. vulgaris* biomass changes throughout seasons and succession and can be affected by soil nutrient and microbial composition (Joosten et al., 2009; Kostenko et al., 2012; Macel et al., 2004). According to Kardol et al. (2006) species succession in the chosen chronosequence may depend substantially on the accumulation of harmful and beneficial organisms in the rhizosphere and not only on abiotic soil properties. Such rhizosphere communities may cause negative soil feedback in early succession, neutral in mid succession and positive feedback to late succession plants (Kardol et al., 2006).

Plant–soil biota interactions may affect the leaf chemical properties and canopy structural properties of a plant, which then possibly translates into a change in spectral reflectance. In a recent greenhouse study we found that such soil biotic effects can, to some extent, affect leaf chemical composition and spectral reflectance (Carvalho et al., 2012). Examining hyperspectral reflectance might provide researchers with further avenues to study plant exposure to ecological processes such as competition, plant diseases, invasiveness and soil biological control of plant abundance, through the, so far, limited temporal scale.

It is essential to understand if the spectral changes that may take place in plants through time could relate to the ecological changes that have already been demonstrated in these plants. As such, we investigated the potential of spectral reflectance to discriminate temporal variation of *J. vulgaris* during the secondary succession stages of abandoned fields. We studied hyperspectral reflectance of both leaves and flowers in order to determine if these two plant organs separately can provide additive information on plant fitness. We tested the hypotheses that: (i) Seasonality and succession stage of *J. vulgaris* are expressed in spectral reflectance of both leaf and flower organs and (ii) the chemical variation resulting from successional and seasonal plant development can be detected in both leaves and flowers.

2. Materials and methods

2.1. Species description

Although native to the Netherlands, *J. vulgaris* is considered a noxious outbreak weed as it is toxic towards humans and livestock and highly dominant in recently abandoned arable fields (Bezemer et al., 2006; Mattocks, 1986). When time of abandonment increases, *J. vulgaris* dominance declines and it largely

disappears from the vegetation (van de Voorde et al., 2012). Its toxicity is mainly due to its pyrrolizidine alkaloids (PA) that are present in all organ types, from leaves to flower heads and seeds. In spring this species has a rosette structure, it flowers via a single central stem throughout the summer, if conditions are favourable (Fig. 1). The circular rosette has basal stalked leaves obovate to pinnately lobed, generally 2–6 cm wide. During early and late summer the rosette leaves senesce and stems develop with pinnate lobed leaves. Flower heads are the characteristic Asteraceae capitulum with bright yellow flowers and green bracts.

2.2. Field sampling

The selected fields belong to a chronosequence of abandoned arable-fields (Kardol et al., 2006). We used information on site characteristics and responses of *J. vulgaris* to soil biota (Kardol et al., 2006; Bezemer et al., 2006; van de Voorde et al., 2011; van der Wal et al., 2006) to selected 6 fields (Table 1). These fields were all located on south Veluwe, Gelderland Province, the Netherlands. Agricultural production had stopped between 5 and 30 years ago and the fields are currently part of a large nature reserve (Bezemer et al., 2006).

Since the fields were of different sizes, in each field we set a W-shaped transect that covered the field central area of 30 m by 100 m. We sampled one plant every 5 m to a total of 20 plants per field. This process was repeated two times throughout the Summer season. As such we covered 2 phenological stages: the flowering and the senescing stage. At each sampling date in all fields, of each individual plant five leaves were measured positioned from base to top of the stem. Five flower heads (the full capitulum, Fig. 1) in the centre of the inflorescence were measured in a lateral perspective to incorporate both the flower petals and the bracts of the capitulum. Both leaves and flowers of each plant were measured still intact and attached to the plant. We used a plant probe and leaf-clip attached to the ASD Fieldspec 3 fieldspectrometer (ASD inc., Boulder CO, USA) to collect the spectral reflectance data. The measured leaves and flowers were immediately collected and stored in ice for chemical extractions in the laboratory thereafter.

2.3. Leaf spectral measurements and processing

Spectral data were collected with an ASD Fieldspec 3 spectrometer with an ASD plant-probe and leaf-clip device (ASD inc., Boulder CO, USA). The instrument has a spectral range between 350 and 2500 nm with 3 nm spectral resolution in the 350–1000 nm and 10 nm between 1000 and 2500 nm wavelengths. The plant-probe was designed for non-destructive data collection from live plants with heat sensitive halogen light bulb (colour and temperature $2901 \pm 10^\circ \text{K}$) and spectral measurement spot size of 10 mm radius. The leaf-clip has a gentle gripping system designed for the plant-probe to hold the sample in place without inflicting damage or removing the sample. Since we were interested in spectral reflectance measurements the black panel face of the leaf-clip was used in each measurement. In each leaf or flower 4 single reflectance measurements were undertaken resulting in a 20-fold composite leaf or flower spectral sample per plant (Ramsey, 1997). All spectral measurements were calibrated with the white reference face of the leaf-clip. The reflectance measurements were offset corrected and its composite average calculated with software ViewSpec Pro 5.6.10 (ASD inc. Boulder, USA). In pre-processing we realized that a technical error occurred with the first season measurements, requiring the rosette leaf measurements to be removed from further analysis.



Fig. 1. Representative leaf and flower heads of *Jacobaea vulgaris*. Top row characterizes early Summer bottom row late Summer.

2.4. Chemical extraction

Chlorophyll a and b (mg g^{-1}), nitrogen (%), carbon (%) and the defence compounds pyrrolizidine alkaloids (mg g^{-1}) were extracted from the five leaves (base to top) while in the flower-heads chlorophyll content was not considered.

The chlorophyll extraction was done using four leaf discs of 10 mm diameter each. The leaf discs were immersed in 3 mL of dymethyl sulfoxide (DMSO) and stored in a dark room for three days at constant room temperature. In a spectrophotometer (Genesys 20 spectrophotometer 4001/4, Thermo Fisher Scientific Inc., Waltham, USA) the 649 nm and 665 nm absorbance (Abs) was measured and chlorophyll concentrations were calculated.

A fine homogenized powder from freeze-dried samples was used for pyrrolizidine alkaloids, nitrogen (N) and carbon (C) estimations. Metal cups of 6 mm diameter were used with 3–5 mg sample powder for combustion-reduction in a C:N analyzer (Thermo flash EA 1112, Thermo Fisher Scientific Inc., Waltham, USA) to estimate C:N percentage.

Pyrrolizidine alkaloids (PAs) were extracted according to Joosten et al. (2009) and quantified as described by Cheng et al. (2011). The PA content was determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS) using a

Waters UPLC system (Waters, Milford, USA) coupled to a Waters Premier XE tandem mass spectrometer (Waters, Milford, USA).

2.5. Statistical analysis

2.5.1. Analysis of variance (ANOVA test) and Tukey post hoc test

One-way ANOVA was performed in the spectral measurements, first with season and succession classes and secondly with season and field as factors. After a significant ANOVA test, a post hoc test Tukey honestly significance difference (Tukey HSD) was performed to test each wavelength in a pairwise manner. This test is conservative for unequal sample sizes and accounts for type I errors by reducing the significance level (α) of each test so that the group-wise type I error rate stays at the chosen level, in this case, $\alpha=0.05$ (Quinn and Keough, 2002). This multiple comparison permits to find those wavelengths that are significantly different between succession groups (young vs medium, young vs late, medium vs late) and seasons with reduced error. The ANOVA analyses were performed for both leaves and flower, using R 2.13.2 for Windows.

2.5.2. Discriminant analysis

While ANOVA tests for differences between groups, discriminant analysis can be applied to generate a combination of features

Table 1

Names of fields selected, code names, time since abandonment (in years) its classified succession class and geo-location. Soil and plant community characteristics are available in (van de Voorde et al., 2012) and (Kardol et al., 2006).

Field	Field code	Time since abandonment	Year of abandonment	Succession class	Latitude ($^{\circ}\text{N}$)	Longitude ($^{\circ}\text{E}$)
Oud Reemst	OR	5	2005	Young	52.02	5.48
Reyerskamp	R	5	2005	Young	52.01	5.47
Mossel	M	15	1995	Medium	52.03	5.45
Nieuw Reemst	NR	20	1990	Medium	52.04	5.47
Wolfheze	W	22	1988	Old	51.6	5.47
Dennenkamp	D	27	1982	Old	52.02	5.48

that maximizes the probability of correctly assigned objects to their defined groups (Quinn and Keough, 2002; Naes et al., 2002). Additionally, the discriminant analyses can be used to classify observations into the groups of interest. In this study we applied quadratic discriminant analysis (QDA) since it does not assume equal within-group covariance. The spectra can be highly collinear and QDA is sensitive to such effects (Naes et al., 2002). To correct for multicollinearity the spectral reflectance was mean-centred and principal component analyses (PCA) was computed (Naes et al., 2002). The resulting PCA scores were then used for performing the QDA. We used 20 principal components as it explained 99.9% of the variance, thus including all the information existent in the original data. Discriminant analysis can have a problem with unequal number of samples per group, overestimating a correct classification, thus prior probabilities were calculated based on the observed group sizes to reduce the random correct classification. The prior probability of the groups describes what is known a priori about the groups to be estimated in the analysis, is based on the Bayes' theorem and is integrated in the discriminant analysis to infer the posterior probabilities (Naes et al., 2002). Success of the classification of the QDA equation was assessed by the quality of the cross-validation confusion matrix and by its success to classify new observations into the groups. The first discriminant function is the combination of variables that maximize the ratio between-group to within-group variation in MANOVA, so that the analysis was considered to test statistical significant differences between the groups (Quinn and Keough, 2002).

By chance classification of samples (also called the error of commission or specificity) is often raised as a problem in spectral data analysis. Different measures of accuracy consider different assumptions and one standardized method to overcome all problems is still challenging (Foody, 2002). By considering the MANOVA statistical test alongside the discriminant analysis we allocate a statistical power to the groups discriminant functions. Highly significant functions should assure that groups compared have lower by-chance classifications. Additionally the Tau index was computed as it provides a standardized measure of the proportional improvement over a model's classification error rate established by chance (Klecka, 1980). The formula applied was:

$$\tau = \frac{N_c - \sum_{i=1}^G P_i N_i}{N_c - \sum_{i=1}^G P_i N_i}$$

N_c is the number of samples correctly classified, N_i is the number of samples in the i th group, N is the total number of samples, G is the number of groups and P_i is the by chance probability of allocating the sample to that group. The groups of interest in this study were the succession classes (Table 1). The QDA was analyzed in Unscrambler X 10.1 and the MANOVA was processed in SPSS 17.0 for Windows.

2.5.3. Partial least square regression (PLSR)

While discriminant analysis tests the possibility for classification of samples into the groups of interest (in this case succession class), with partial least square regression (PLSR) we tested if specific spectral band data can be used as predictors of chemical concentrations in individual samples (Naes et al., 2002). Therefore we examine the linkage between the sample chemical content and its spectral signature. The cross-validation procedure selected was 'leave-one-out' sampling that iteratively generates regression models with 1 random sample reserved for validation of the model. This was done in a training-set with approximately 70% of the samples to determine the optimal number of factors and lowest root mean square error (RMSEcv) of cross-validation. The accuracy of the model for prediction of chemical concentrations was assessed in terms of minimum root mean square error of prediction (RMSEp)

and the highest coefficient of regression (r^2) of the test-set (i.e. the reserved 30% of the samples). The entire procedure was done in Unscrambler X 10.1 for Windows.

3. Results

3.1. Spectral reflectance differences in leaf and flowers

It was possible to significantly differentiate between leaves and flowers by spectral reflectance patterns (Figs. 2 and 3). The analysis of *J. vulgaris* leaves and flowers revealed statistical significant differences, which were more prominent in the flower spectral reflectance than in leaves. Both leaf and flower spectral reflectances showed variations in relation to succession stage and season.

Hyperspectral reflectance of leaves from plants in old succession stages was significantly different from young succession stages both in the visible range (500–650 nm) and in the inflection between the visible and near-infrared (700 nm region) (Fig. 3). Flowers of plants from younger fields reflected significantly less in the 500–650 nm range than flowers from medium or old succession stages (Fig. 3). No significant differences were found between flower reflectance of medium and old succession classes ($p > 0.05$).

There were differences between early and late summer in the red edge area (690–710 nm region) of leaves (Fig. 3, $p < 0.05$). Flower spectra in early and late summer were significantly different through the entire visible and infra-red region (Fig. 3, $p < 0.05$).

3.2. Succession class discrimination

It was possible to discriminate successional classes with high accuracy (of cross-validation) for both flower and leaf spectral reflectance patterns. The successional classes underlying dimensions of the cross-validated model were highly significant ($p < 0.001$) in explaining differences. In addition, Tau results indicate that the classification analysis obtained between 90% and 100% fewer errors than what would be expected by chance.

The succession classes discrimination analyses with both seasons together resulted overall classification accuracy, by the quadratic discriminant analysis, of 92.3% for the leaf cross-validation model and 94.4% for the flowers (Table 2). Even so, succession had only moderate prediction accuracy in the test-set with 56.1% for leaf and 65% for flower correct prediction of unknown samples.

The discrimination analyses with early and later Summer separately resulted in 100% overall correct classification in the cross-validation model, for both leaves and flowers (Table 3). However early Summer provided a low prediction accuracy for the validation samples for flowers (32.5%) and leaves (50%) while late Summer was low to moderate with 52.6% for leaves and 60% for flowers correct prediction for the validation samples.

3.3. Chemical content estimation in leaves and flowers

There were significant differences in chemical content between the seasonal and successional classes (Figs. 4 and 5). The PA concentrations were affected by both season and succession stage, whereas nitrogen was significantly affected only by season. This pattern was found in both leaf and flower organs, and partial least square regression (PLSR) was successful in the estimation of several chemical concentrations.

The most successful foliar estimates occurred in the primary compounds, nitrogen and chlorophyll (Table 4). The prediction of unknown samples by such models resulted in moderate correct estimations of foliar content with root mean square errors of

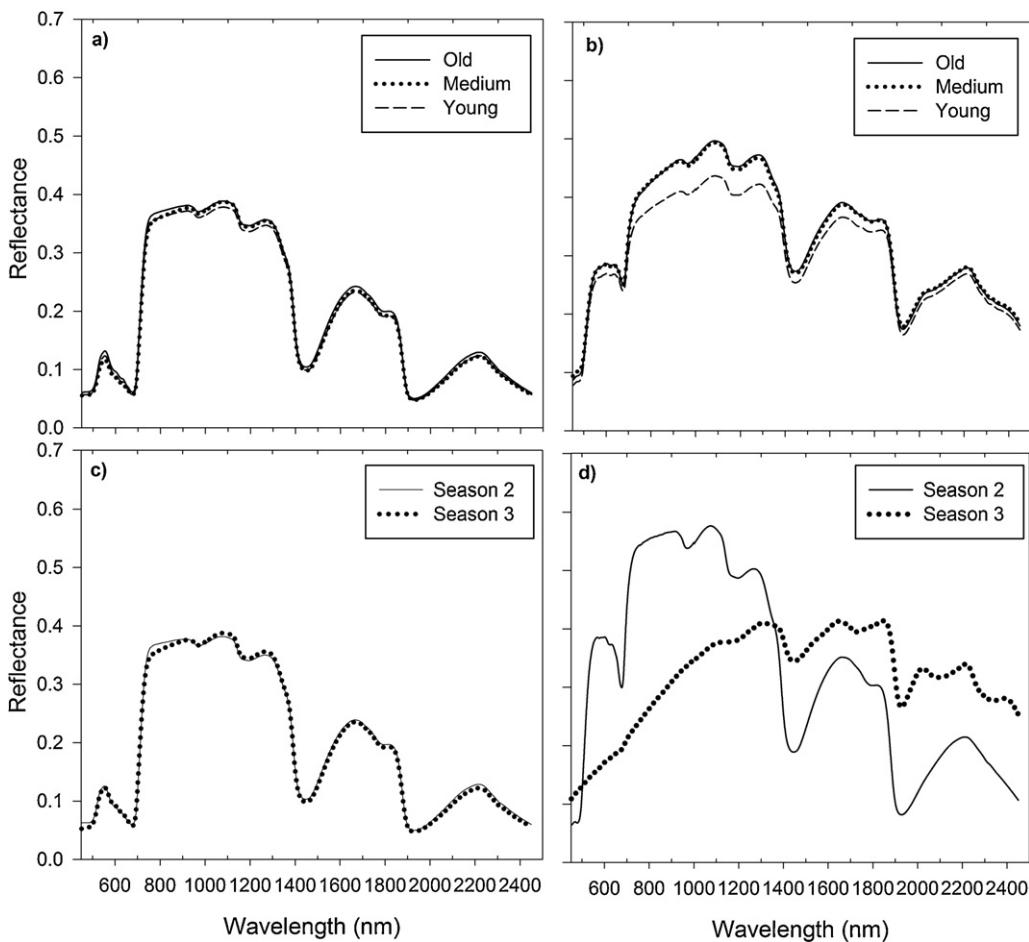


Fig. 2. Mean spectral reflectance of (a) succession class in leaves, (b) succession class in flower heads (c) seasons in leaves and (d) seasons in flowers heads.

prediction (RMSEp) in the 12–15% range of the mean. The PLSR failed prediction of foliar PA content (Table 4).

While the most accurate estimations of leaf chemicals concerned the primary compounds, in flowers the highest accuracy

was obtained for estimates of the defense compounds (Table 4). Using PLSR we were able to estimate unknown samples between 38 and 61% accuracy. The best estimation accuracies were 23% and 29% of the mean for total PA and N-oxides, respectively.

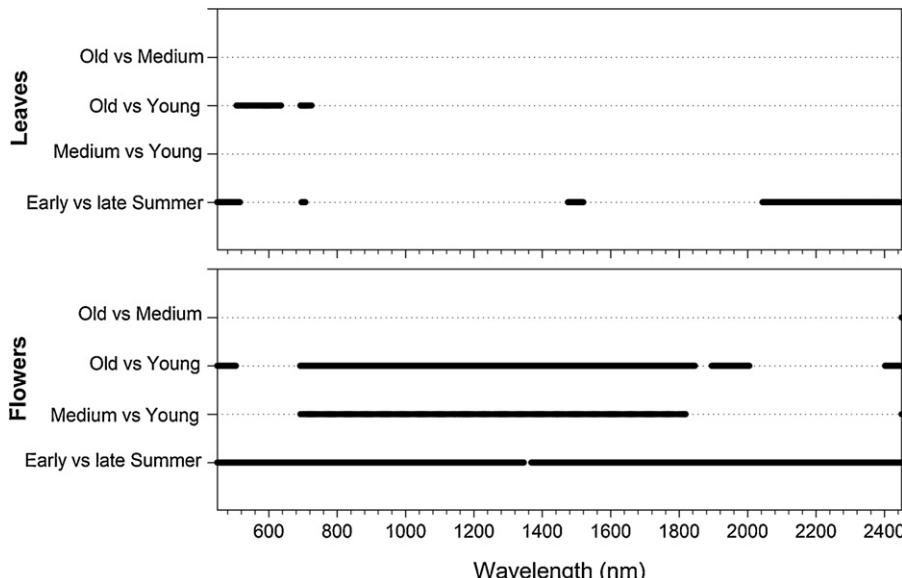


Fig. 3. One way ANOVA and Tukey HSD test results for leaf and flower reflectance measurements. Dark circles indicate wavebands that were significantly different ($p < 0.05$) in each pair comparison. Early Summer vs late Summer – early Summer and late Summer comparison; Old vs Young–Old succession vs Young succession fields. Old vs Medium–Old and Medium succession comparison; Medium vs Young–Medium and Young succession comparison.

Table 2

Confusion matrix of the quadratic discriminant analysis with both seasons considered. Succession stage was used as the discrimination group. The first 20 principal components of the spectral reflectance were used in the QDA. The bold number along the diagonal represents the number of correct classified samples. The results display the cross validation (leave-one-out) and separate test set for classification of unknown samples for both leaf ($\tau = 0.88$) and flower heads ($\tau = 0.92$). All discriminant functions were highly significant ($p < 0.001$). In brackets is the number of samples used in each dataset.

Crossvalid (168)			Predict (59)			
Old	Medium	Young	Old	Medium	Young	
Both seasons						
Leaf						
Old	54	2	2	14	6	5
Medium	1	47	0	3	9	4
Young	3	5	54	5	4	9
Crossvalid (178)			Predict (60)			
Old	Medium	Young	Old	Medium	Young	
Both seasons						
Flower						
Old	57	2	3	12	2	4
Medium	2	57	2	4	14	2
Young	0	1	54	4	4	13

Overall accuracy in cross-validation was 92.3% in leafs, 94.4% in flowers with predicted accuracy of 56.1% and 65% respectively.

Tertiary amines presented the highest error with 61.6% of the mean. Contrary to the foliar PLSR, predictive models for flower contents failed to estimate primary compounds (Table 4). The most accurate PLSR models, both for leaves and flowers, consistently selected the spectral regions known from literature (data not shown), such as reported by Curran (1989), Kumar et al. (2001) and Carvalho et al. (in press).

4. Discussion

In this study of temporal variation in the hyperspectral reflectance of *J. vulgaris*, two main effects were revealed: (i) there are successional and seasonal variations in spectral reflectance of leaves and flowers (ii) the defence chemical content of flowers can be detected through hyperspectral reflectance.

Table 3

Confusion matrix of the quadratic discriminant analysis per season. Succession stage was used as the discrimination group. The first 20 principal components of the spectral reflectance were used in the QDA. The bold number along the diagonal represents the number of correct classified samples. The results display the cross validation (leave-one-out) and separate test set for classification of unknown samples for both leaf ($\tau = 1$) and flower heads ($\tau = 1$). All discriminant functions were highly significant ($p < 0.001$). In brackets is the number of samples used in each dataset.

Crossvalid (74)			Predict (38)			
Old	Medium	Young	Old	Medium	Young	
Early Summer						
Leaf						
Old	25	0	0	6	2	4
Medium	0	25	0	1	8	3
Young	0	0	24	6	2	5
Crossvalid (80)			Predict (40)			
Old	Medium	Young	Old	Medium	Young	
Early Summer						
Flower						
Old	27	0	0	3	3	1
Medium	0	27	0	6	5	2
Young	0	0	26	5	4	5
Overall accuracy in cross-validation was 100% in leafs and flowers with predicted accuracy of 50% and 32.5% respectively.						
Crossvalid (74)			Predict (38)			
Old	Medium	Young	Old	Medium	Young	
Late Summer						
Leaf						
Old	25	0	0	6	2	4
Medium	0	25	0	1	8	3
Young	0	0	24	6	2	5
Crossvalid (78)			Predict (40)			
Old	Medium	Young	Old	Medium	Young	
Late Summer						
Flower						
Old	25	0	0	10	2	2
Medium	0	27	0	5	6	2
Young	0	0	26	3	2	8

Overall accuracy in cross-validation was 100% in leafs and flowers with predicted accuracy of 50% and 52.6% respectively.

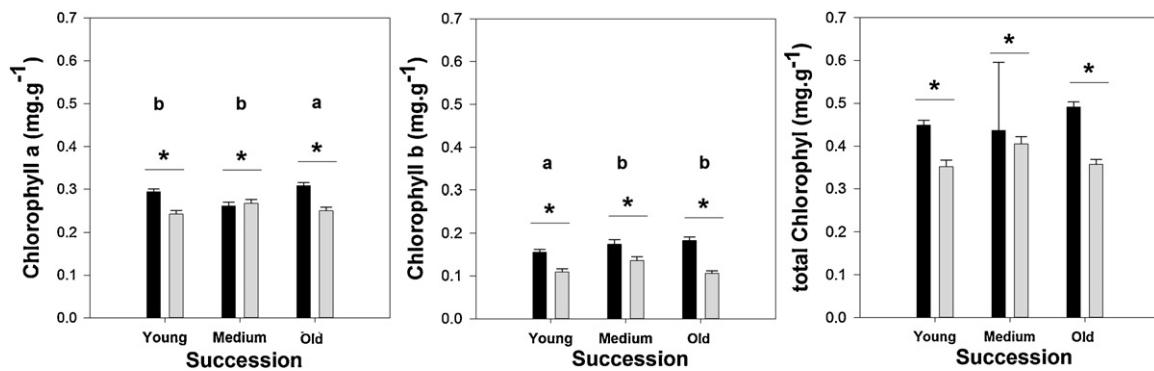


Fig. 4. Chlorophyll content per succession stage (young, intermediate, old) in leaves, for both early (black bars) and late Summer (grey bars). Asterisk represents significant differences between seasons. Letters represent significant differences between succession categories ($p < 0.05$). Error bars are standard errors.

We had expected seasonal differences in hyperspectral reflectance between flowers throughout the whole visible and infrared range, as in early Summer the flower capitula were still yellow, whereas in late Summer flowers were replaced by dry white papus. Such clear differentiation in leaves was far less obvious. This lack of full differentiation of spectral information between leaves from different seasons might be influenced by spectral measurements done only in live leaves. Brown and dead leaves have not been considered in this study. Another limitation to this seasonal study is the lack of the Spring season data, when plants were in the rosette stage. This lack of data is due to an initial technical problem, so that we were unable to include this early seasonal stage in our analyses. Nevertheless, we believe that Spring season data could very well improve the discrimination ability of our approach, as it is a fully different ontogenetic stage of the life cycle of *J. vulgaris*. However, further tests are necessary.

The successional class of old field succession defined by Kardol et al. (2006) resulted in a successful differentiation using the hyperspectral information of flowers. This suggests that flower composition is more sensitive to field ageing processes than that of leaves. The hyperspectral reflectance of leaves showed significant differences between old and young succession fields in the visible region, suggesting that differences in the photosynthetic process may occur at larger temporal scales than usually thought. The spectral changes in the visible wavelengths are supported by the significant differences found in concentration of chlorophyll a and b between these succession groups. The resulting spectral

changes between plants found in the present study can, at least to some extent, be related to the chemical changes in the plants. This suggests that changes in plant chemical properties during succession possibly translate into the changes in spectral reflectance and that plant successional position in the field might be identified through analysis of hyperspectral reflectance data. The results support, to some extent, our hypothesis as the reflectance by flowers was sensitive to succession stages, and both leaf and flower spectral patterns reflected, to some degree, the plant chemistry.

Our study suggests that succession category affect, albeit slightly, the plant spectral reflectance. The high cross-validation results in our study and significant explained variation by the discrimination functions suggest that even within species the statistical significant discrimination of groups (succession) is possible. However the natural continuum range of variation has some level of erroneous prediction of new samples due to groups that overlap. Since the accuracy of our cross-validation discriminant analysis is similar to results from some other studies (Asner and Martin, 2011; Ramoelo et al., 2011b), we suggest that there should be caution in the interpretation of the discrimination power based solely on cross-validation models. A test set for prediction of new samples should always be considered due to the continuum variation naturally occurring in field conditions. The discrimination accuracy by the spectra of the plant organs was also season dependent, whether we group the season or use them individually, but in general, succession class discrimination achieve similar levels of accuracy. We demonstrated that chemical concentration

Table 4

Species chemical concentration estimations by partial least square regression using leaf or flower spectral reflectance signatures. r^2 calib represents r^2 of model calibration, r^2 cv represents r^2 of cross-validation (leave-one-out) and r^2 predict represents how good is the fit of the cross validated model to predict new samples from a separate test-set. RMSEp is root mean square error of prediction and %RMSEp is the error as a percentage of the mean value of the chemical concentration. Num.Factors indicates the number of factors selected by the model. Dash represents PLSR models that failed prediction and consequently the RMSE presented is of cross-validation. PA – pyrrolizidine alkaloids, which act as defensive components in *J. vulgaris*.

Leaf	Mean	St. dev.	r^2 calib	r^2 cv	r^2 predict	RMSEp	%RMSEp	Num.Factors
Nitrogen (%)	2.30	0.58	0.73	0.66	0.62	0.29	12.77	9
Carbon (%)	42.77	1.76	0.22	0.13	0.06	1.89	4.43	5
Chlorophyll a (mg g^{-1})	0.28	0.05	0.59	0.50	0.26	0.04	14.80	7
chlorophyll b (mg g^{-1})	0.15	0.06	0.65	0.60	0.60	0.03	21.38	5
chlorophyll total (mg g^{-1})	0.42	0.09	0.68	0.62	0.37	0.06	15.13	5
Total PA (mg g^{-1})	0.69	0.27	0.02	0.00	–	0.30	42.96	1
Tertiary-amines (mg g^{-1})	0.17	0.18	0.02	0.00	–	0.15	85.96	1
N-oxides (mg g^{-1})	0.62	0.29	0.01	0.00	–	0.30	48.95	1
<i>Flower</i>								
Nitrogen (%)	2.09	0.35	0.26	0.25	–	–	–	1
Carbon (%)	44.50	3.37	0.06	0.02	–	3.45	–	1
Total PA (mg g^{-1})	0.65	0.26	0.73	0.67	0.61	0.15	23.55	3
Tertiary-amines (mg g^{-1})	0.19	0.16	0.41	0.40	0.38	0.11	61.62	2
N-oxides (mg g^{-1})	0.60	0.25	0.63	0.56	0.47	0.18	29.77	3

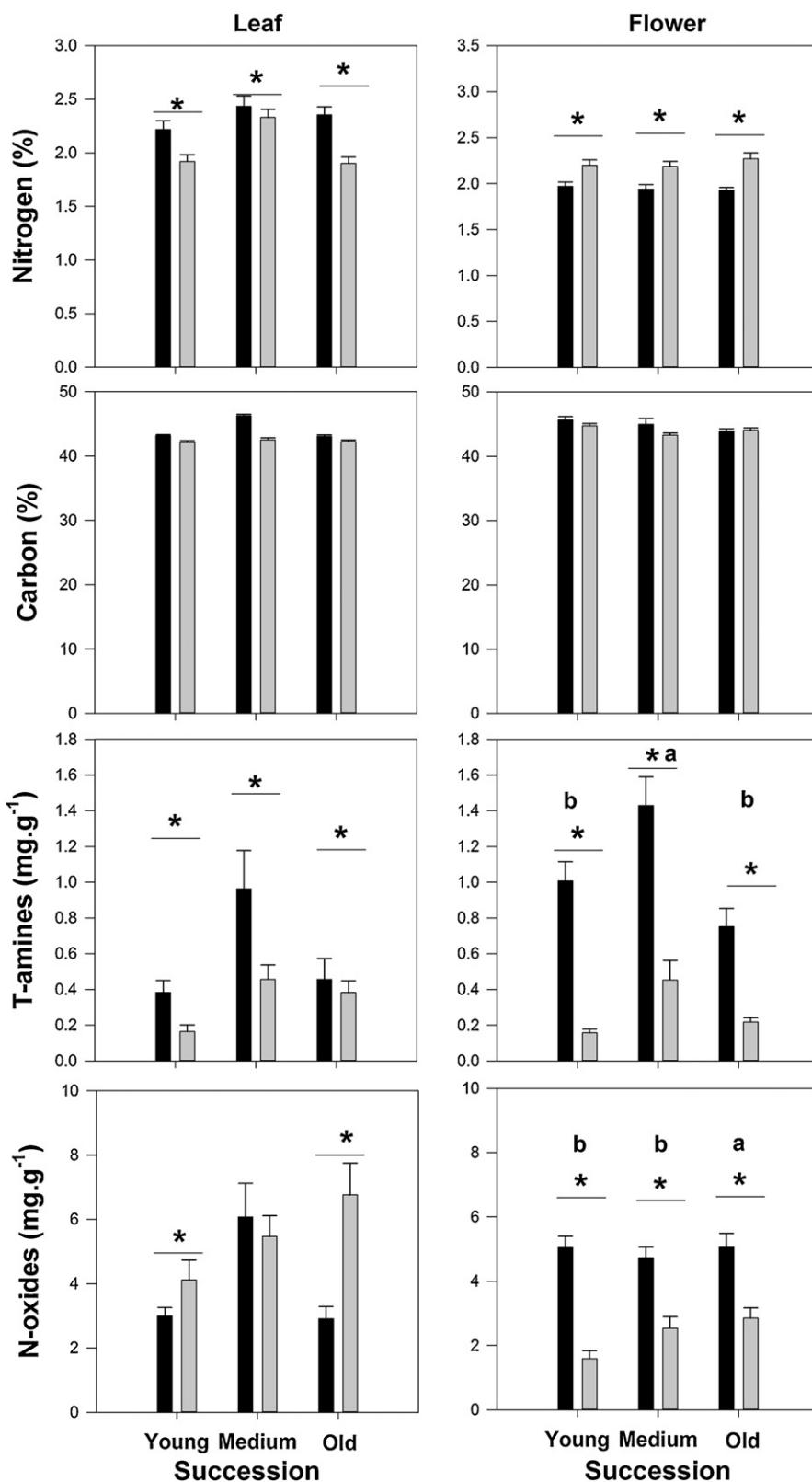


Fig. 5. Chemical content (primary and secondary) per succession stage (young, intermediate, and old) in leaves and flowers, for both early (black bars) and late Summer (grey bars). The asterisk represents significant differences between seasons. Letters represent significant differences between succession categories ($p < 0.05$). Error bars are standard errors.

estimation using spectral data is possible for both leaves and flowers. The cross-validation results obtained for foliar estimation of nitrogen and chlorophyll were consistent with earlier studies, including the spectral features selected for the models (Knox et al.,

2010; Asner and Martin, 2011; Ramoelo et al., 2011b; Darvishzadeh et al., 2008; Curran et al., 2001). Its accuracy provided a moderate predictive ability of chemical concentration in new leaf samples, showing that even in controlled field measurements such chemical

concentration estimations by hyperspectral reflectance are challenging. The flower estimation results were the reverse of results on leaves. Nitrogen contents could not be predicted in flowers, whereas estimations of PA compounds were moderately accurate. Concentration wise both flowers and leaves were relatively similar in nitrogen concentration variation. The differences between flowers and leaves were therefore unexpected. Both Asner and Martin (2008) and Kokaly and Clark (1999) highlighted that vegetation structure can affect spectral features and influence chemical detection. It could be that the flower structure (i.e. the capitulum shape) affects the spectral features associated with nitrogen estimations resulting in such estimation inconsistencies between organs. The flower structure combined with lower water content in flower could have aided the features associated with PA estimations. However, using the same technology, Carvalho et al. (in press) were able to estimate foliar PAs with moderate accuracy in laboratory conditions. Other studies might be necessary to analyze what could cause such leaf and flower predictive differences. Nevertheless, the two plant organs can affect the chemical estimation by spectral features differently. This is important to study further as upscale and temporal studies are foreseen in remote sensing.

Differences in *J. vulgaris* successional position might be detected by spectral reflectance and this old-field chronosequence has been proposed to be driven, at least in part, by soil biota (Kardol et al., 2006; van de Voorde et al., 2012). Our results show that spectral reflectance could add information to temporal studies in the field on complex ecological processes driven by for example cryptobiota that influence plant performance, which go beyond the impact of large vertebrate herbivores. Moreover, we show that different plant organs may vary in an organ-specific way during secondary succession. More insight into plant–soil interactions and their impacts on spectral reflectance patterns might be considered as having potential for studies on biological control of invasive species or soil-borne diseases. An important issue to solve is the multiple information aspect of spectral signals in imagery. In imagery both leaf and flower information will be detected during flowering season. What the spectral signal of a plant as a whole may tell about interactions, successional situation, its adaptation, etc., needs to be established in subsequent studies to improve the development of accurate extrapolations to imagery.

5. Conclusion

We addressed the capacity of spectral reflectance to discriminate succession stages (by age category) and season as well as the importance of the plant organ for *J. vulgaris*. There are important temporal aspects of plants affected by (a)biotic factors relevant to ecology. The cross-validation models showed potential for detecting seasonal and successional differences in plant performance using hyperspectral reflectance patterns. However, the predictive accuracy of new samples was low. The results were organ dependent and spectral overlap may be the main reason for some of the lowest outcomes. Yet the spectral variation in both flowers and leaves is supported by the variation in chemical concentration of *J. vulgaris*. Defence compounds could be estimated more reliably in flowers, whereas of leaves primary compounds could be predicted best. As such we suggest that remote sensing studies should consider the effect not only of phenology but also of different organs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jag.2013.01.005>.

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