Wetlands Ecology and Management **12:** 353–363, 2004.

Colour-based estimation of rhizome age in *Phragmites australis*

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Received 18 October 2002; accepted in revised form 6 June 2003

Key words: Branching hierarchy, Bulk density, Colour key, Euclidian distance, Munsell colour charts, Rhizome age, Rhizome colour, Spectral reflectance

Abstract

The colour of different age groups of *Phragmites australis* (Cav.) Trin. ex Steudel rhizomes was studied from April through October 2000 at approximately one-month intervals to propose a more efficient method to identify the rhizome age based on the Munsell colour-order system. Seven rhizome age-classes were recognized, from <1 to 6 years old, based on descriptions published in the scientific literature. During April and May sampling, spectral reflectance between 400 and 700 nm of different rhizome ages was measured at 10 nm intervals, using a spectral colorimeter. Rhizomes of different ages were assigned colours by selecting one/two shortest Euclidian distances between the mean spectral reflectance of each rhizome age category and the Munsell colours on the four-dimentional subspace, made by Principal Component Analysis of the spectral reflectance data of 1289 Munsell colours. The Munsell colour for new to six-year-old rhizomes changed from yellow to yellow-red, and the value decreased from new to six-year-old rhizomes, indicating a darkening with ageing. The age of rhizomes collected from April through October was estimated using the colour key, in addition to the age attribution based on branching hierarchy. Between 87% and 100% of the rhizomes attributed to a certain age class based on branching hierarchy were assigned to the same age class using colours during all sampling dates. There was a strong correlation ($r = +0.96$) between rhizome age estimated by branching hierarchy and colour. At each sampling, bulk density, an indicator of rhizome storage levels, measured as a verification of age identification, varied among the age categories indicating distinct differences in storage levels. These results confirmed that rhizomes of a specific age category could be assigned a distinct colour, which remains more or less unchanged throughout the growing season. Thus, colour can be used as a primary criterion in the estimation of the age of *P. australis* rhizomes.

Introduction

Like many emergent macrophytes, *Phragmites australis* (Cav.) Trin. ex Steudel is a rhizomatous perennial plant, largely depending on rhizomes for its survival and spread (Chapin et al. 1990). Studies of reed rhizomes are complicated by the fact that the rhizomes live for several years and their age affects numerous rhizome characteristics, such as respiration (Cizkova and Bauer 1998) or starch and sugar accumulation (Fiala 1976). The studies concerning the age-specific storage/metabolic characteristics of rhizomes are of importance to understand fully the ecophysiological mechanisms of *P. australis* (Graneli et al. 1992). With few exceptions (Cizkova and Lukavska 1999; Klimes et al. 1999; Cizkova and Bauer 1998; Fiala 1976), most of the previous studies done on carbohydrate

storage, nutrient contents and bulk density of *P. australis* rhizomes, have not taken into account the differences among age categories. One of the reasons why age was neglected was certainly the difficulty of its estimation, which probably discouraged many researchers from including this parameter in their studies.

Usually, identification of different age categories of *P. australis* rhizomes is a tedious and a time-consuming process associated with branching hierarchy supported by condition of scale leaves, attached roots and other related parameters. It is well known that rhizome colour becomes darker with age and may be site-specific. Even though colour has been used as a comparative (Klimes et al. 1999) and secondary criteria (Cizkova and Lukavska 1999) in determining the rhizome age by branching hierarchy, its potential, as a primary selector has not been subjected to a quantitative investigation.

The colour of an object is a sensation, which is produced in the brain and thus is hard to define. But the spectral reflectance of an object can be measured by a spectral colorimeter, thus enabling to define the colour in an unique way (Kaiser and Boynton 1996). Earlier studies (Hauta-Kasari et al. 1999; Jaaskelainen et al. 1990) that compared the subspace of spectral reflectance of natural living objects, such as plants, flowers, etc. with the subspace calculated from the spectral reflectance of Munsell colour chips showed strong correlations. Therefore, Munsell colour-order system, which includes most of the natural colours human sensation can recognize, can reliably be used as a general standard to express the colours of living objects found in nature. Therefore, the present study sought to investigate the colour of different age groups of *P. australis* rhizomes to propose a more efficient method to identify the rhizome age based on the Munsell colour code. It has been shown that rhizomes of different age categories have different characteristics, such as storage and respiration (Cizkova and Lukavska 1999; Klimes et al. 1999; Cizkova and Bauer 1998). Therefore, rhizome bulk density (dry mass per fresh rhizome volume), an easily measured parameter proportional to the quantity of non-structural polysaccharide reserves in the rhizome, is measured for rhizome segments of different ages to countercheck and verify the identification.

Materials and methods

Study area

The study was conducted in a wetland portion of Akigase Park, near the Arakawa River in Saitama City, Japan (35° 51′ N, 139° 39′ E). The park, located on the flood plain of the Arakawa River, is a nature reserve covering some 500 ha adjacent to the river and comprises many such wetland areas. The study site covering about 0.1 ha was dominated by a monospecific and more or less homogeneous (shoot height and stem distribution) stand of *P. australis*. The *P. australis* stand was more than 10 years old and appeared to be in a stabilized condition.

Rhizome sampling

Three replicate samples of rhizomes and roots were taken at each sampling date by excavating soil under a surface area of 0.125 m^2, to a minimum depth of 0.6 m. Sampling was always performed in a visually homogeneous, monospecific area of uniform shoot density and age. Plant samples were harvested on April 28, May 10, June 4, July 18, August 1, September 18, and October 24, 2000.

Plant materials were cleaned of soil with a pressurized water spray. When cleaning the rhizome mat, care was taken to preserve the interconnected rhizome branches. The washed rhizome mat was then carefully separated into clusters of interconnected rhizome branches.

Rhizome dating

Only the undamaged rhizome clusters with interconnected rhizome branches were used in rhizome age determination and subsequent bulk density, ρ_{rhiz}, determinations. These clusters were blotted and the branches were tentatively dated according to the position in the branching hierarchy, a method modified from Cizkova and Lukavska (1999) and Klimes et al. (1999). The condition of the stems attached to vertical rhizomes (a vertical rhizome attached to a green shoot is one-year-old, etc.) and the condition of the nodal sheaths (intact and tightly covered in newly formed rhizomes, loosely attached or partly disintegrated in one- to two-year-old rhizomes and absent in more than

three-year-old rhizomes) were used as supplementary criteria. Rhizomes up to six years old were identified from the stand.

1. *New rhizomes* (younger than one year): Rhizomes bearing a terminal bud formed in the current year, tightly covered with scale leaves, present only after June sampling.
2. *One-year-old rhizomes* (formed in the previous year): Rhizomes bearing a green shoot in the terminal position, with preserved scale leaves, roots missing during the early growing season and with sparse roots during the late season.
3. *Two-year-old rhizomes*: Rhizomes bearing a light brown dead culm in the terminal position, usually attached to a new or one-year-old rhizome, sometimes with scale leaves, generally with adventitious roots. Such rhizomes had rusty coloured spots, which were removable upon washing with water while rubbing.
4. *Three-year-old rhizomes*: Rhizomes generally bearing a dark brown or almost black dead culm in the terminal position, usually attached to a new, one- or two-year-old rhizome, generally with adventitious roots.
5. *Four-year-old rhizomes*: Rhizomes generally bearing a black or rotted dead basal part of a culm in the terminal position, usually attached to a new, two- or three-year-old rhizomes, generally with large roots.
6. *Five-year-old rhizomes*: Rhizomes more frequently attached to three- or four-year-old rhizomes than two-year-old rhizomes, some times with large attached roots.
7. *Six-year-old rhizomes*: Rhizomes generally attached to four- or five-year-old rhizomes, often with longest nodal lengths, usually with sparsely or no attached roots.

Analysis of rhizome colour

The Munsell colour-order system consisting of 1289 paper colour chips is a way of precisely specifying colours and showing the relationships among colours. Colour of any surface can be identified by comparing it to the chips, under proper illumination and viewing conditions. Therefore, Munsell colour code which is classified according to hue (Munsell hue), lightness (Munsell value) and saturation (Munsell chroma) was used in this study to assign colours to different rhizome age categories.

During April and May sampling, spectral reflectance in the range between 400 and 700 nm of different rhizome age categories were measured at 10 nm intervals, using a spectral colorimeter (Minolta-CM-3600d). The wet samples were wiped with a cotton cloth just before the measurements to prevent any white rust formation on the rhizomes older than four years. Freshly dug, undamaged rhizomes were always used in the analyses to prevent changes in colour that biased the results. Low new rhizome biomass observed in June delayed the spectral reflectance measurements of new rhizomes until July even though, the formation started as early as June. Also a database to form a subspace to analyse rhizome data was compiled by measuring the spectral reflectance (in the range of 400–700 nm) of Munsell colour chips from Munsell book of colour-matte finish collection (Munsell colour, Baltimore, MD, 1976). *P. australis* rhizomes of different ages were categorized according to colour by comparing their mean spectral reflectance with the Munsell database on a subspace (Oja 1983). The subspace to expand these data was constructed by Principal Component Analysis (PCA). Original spectral distributions of Munsell colour chips are described as a 31-dimensional vector. According to the PCA, resultant eigenvectors are orthogonal to each other and most statistically important characteristics of the original data are included in these few upper eigenvectors. In the present study, a resultant subspace consisting of four upper eigenvectors guaranteed fidelity of over 99.8%. Therefore, the four-dimensional subspace (Figure 1) was used to expand the spectral reflectance of every Munsell colour and age-specific rhizome samples. Each age category was assigned a colour range by selecting the two shortest Euclidian distances between each rhizome age category and Munsell colours in the subspace. Euclidian distances to the other possible colours being too far, the new rhizomes were assigned only one colour.

Measurements in April and May yielded similar results. Past experience had also shown that the rhizome colour remains more or less unchanged throughout the season. Therefore, it was decided to construct the colour key using the April data.

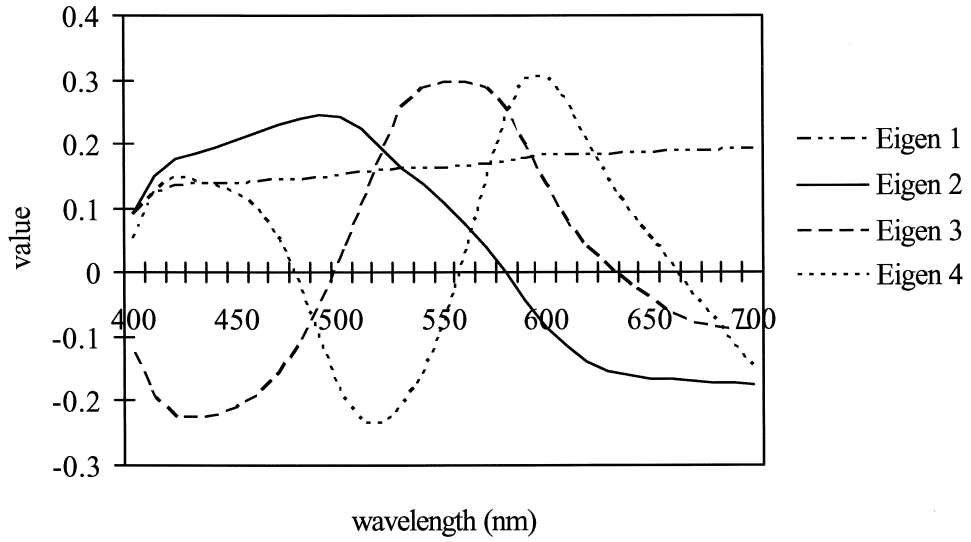

Figure 1. The first four eigenvectors based on the spectral reflectance data of Munsell colours calculated by PCA.

At each sampling, including April and May, the percentage of rhizome segments of a given age according to the branching hierarchy, falling within the corresponding age group according to the colour key based on Munsell colour code, was also calculated as verification. The surface of the rhizome samples was completely dry and free of any moisture when the samples were measured in the spectral colorimeter. Therefore, the same conditions were maintained when the samples were visually compared with the colour key based on Munsell colour code.

Rhizome bulk density determination

Rhizome bulk density (ρ_{rhiz}), an easily measurable parameter and a good indicator of the current content of reserve substances, such as total non-structural carbohydrate was determined to confirm the identification of *P. australis* rhizome age. At each harvest date, ρ_{rhiz} of rhizome segments of different ages was measured. Using a sharp knife to prevent damage, rhizome segments bearing two undamaged nodes at either extremity of an internode were excised from undamaged rhizome branches. On each sampling date, ρ_{rhiz} of some 30–40 intact internodes from each age category was measured. However, the numbers of rhizome internodes at the early stage of new rhizome formation and of six-year-old rhizomes, especially after May, were limited to 15–25, due to the low relative biomass of these age categories. The volume of a rhizome internode in milliliters was measured by water displacement when a fresh intact rhizome internode was completely immersed in the water with the aid of a needle (V). Dry mass of the rhizome internodes in grams was obtained after drying them to a constant mass at 85°C (W); ρ_{rhiz} of rhizome internode was then defined as dry mass per unit volume of fresh rhizome [W/V (mg DW mm^{-3})].

Statistics

The bulk density data were evaluated using analysis of variance (ANOVA) with Tukey's multiple comparison as a *post-hoc* test. Bartlett's test was used to test the homogeneity of variances. An unpaired *t*-test was used to evaluate the differences between two independent means. The reflectance data were analysed using PCA.

Results

In the Kanto region of Japan, where the study site is located, the lifespan of *P. australis* shoots is one growing season. Shoots start growing in early April and completely die towards December. The absence of two branching levels of rhizomes

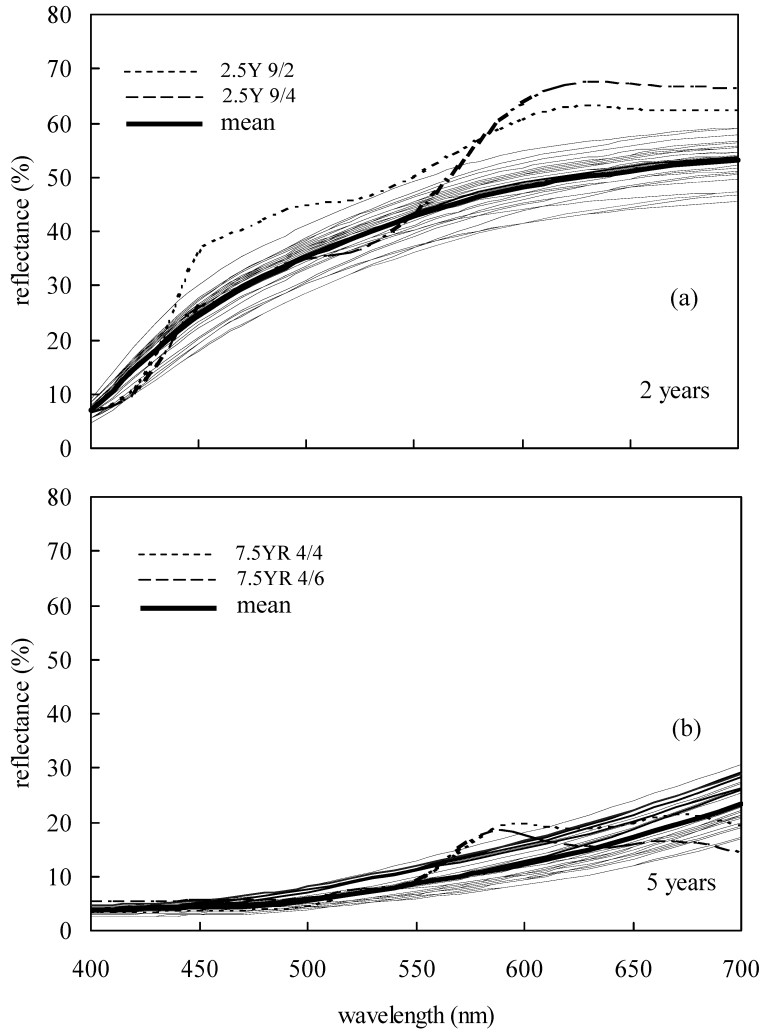

Figure 2. Spectral reflectance (%) characteristics of (a) two- and (b) five-year-old rhizomes with corresponding Munsell colours measured between 400 and 700 nm at 10 nm intervals.

bearing green shoots indicated that rhizomes did not branch twice a year. Similar observations have been made in some Central European stands (Cizkova and Lukavska 1999). Some mid-summer formation of shoots was observed (starting from August, about 12 shoots per m^2) but these summer shoots belonged to the same branching level as the rhizomes bearing green shoots formed earlier in the spring. We therefore concluded that each branching level typically corresponded to one year of age.

Figure 2 shows the spectral reflectance characteristics of two- and five-year-old rhizomes together with that of corresponding Munsell colours. Even though, both rhizome age categories showed low reflectance values, falling below 10% at lower wavelengths, they showed a marked variation at higher wavelengths. Mean reflectance of two-year-old rhizomes exceeded 50% at 700 nm while that of five-year-old was only 23%, showing the darkening of rhizome colour with age (i.e., lighter colour surfaces reflect higher percentage of light while dark colour surfaces reflect lesser percentage of light). Similar reflectance patterns could be observed with the other rhizome age categories, younger rhizomes reflecting higher

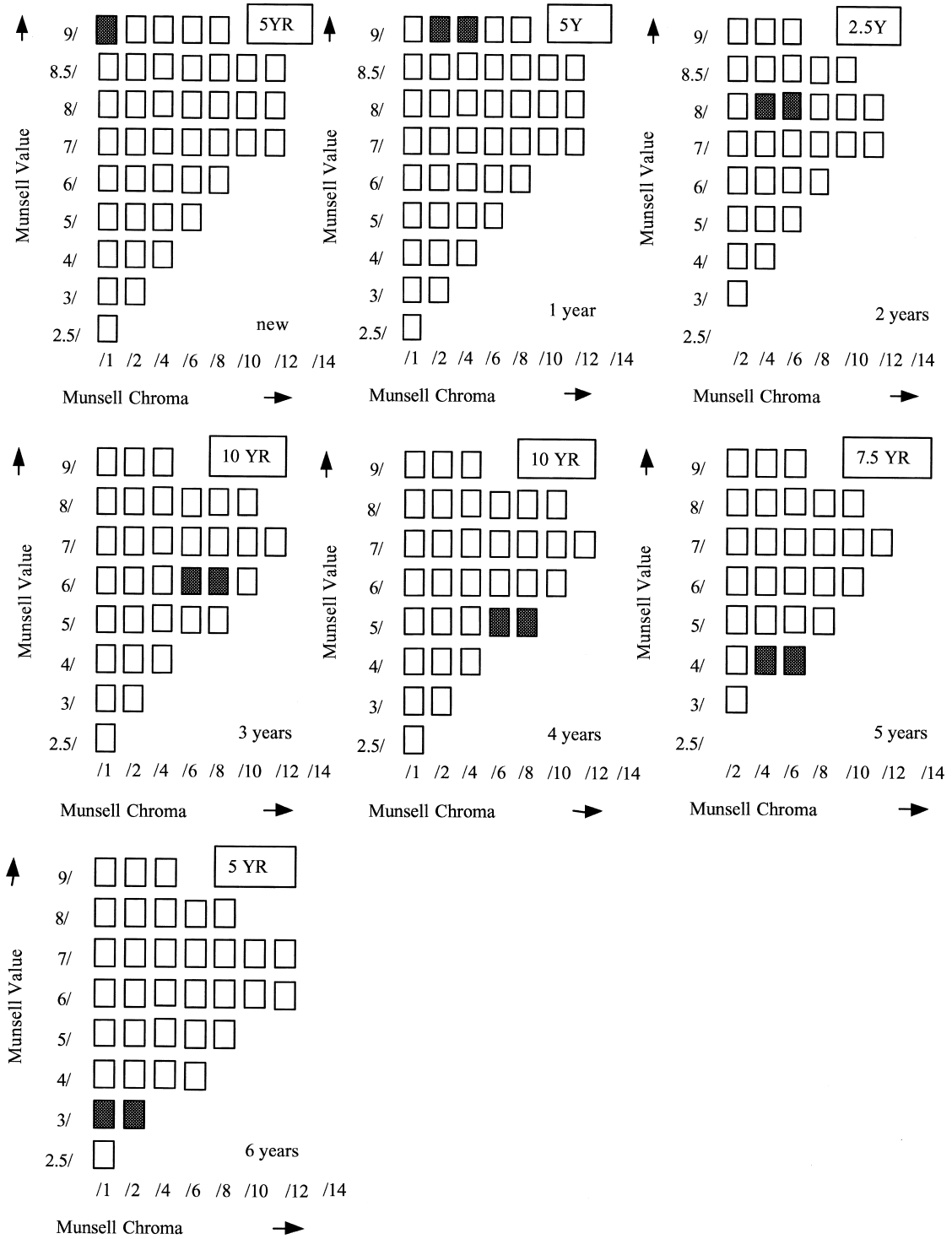

Figure 3. Colour key according Munsell book of colour-matte finish collection (Munsell colour, Baltimore, MD, 1976) for the identification of *P. australis* rhizomes at Akigase park, Saitama, Japan (Munsell colour is based on steps of equal visual perception with any colour being defined as a point within the three-dimensional Munsell colour space. The system's attributes are Munsell Hue, Munsell Chroma and Munsell Value which correspond to perceived hue, chroma and lightness, respectively).

Table 1. The percentage of rhizome segments attributed to a certain age class based on the position in branching hierarchy, falling within the same age class, proposed by the colour key based on Munsell colour code (estimated by visual comparison).

		Years					
Date	New	1	2	3	4	5	6
28.04.00	–	100	97	96	94	95	100
09.05.00	–	97	95	98	95	94	100
02.06.00	–	98	92	93	91	91	96
03.07.00	100	96	97	92	93	87	93
01.08.00	100	95	94	93	89	90	90
07.09.00	100	91	91	88	90	89	92
24.10.00	100	89	92	88	89	89	90

percentage of light compared with their older counterparts.

The rhizome colour of different age groups as defined by the Munsell book of colour-matte finish collection is shown in Figure 3. The Munsell colour range for new to six-year-old rhizomes was 5YR 9/1; 5Y 9/2, 9/4; 2.5Y 8/4, 8/6; 10YR 6/6, 6/8; 10YR 5/6, 5/8; 7.5YR 4/4, 4/6 and 5YR 3/1, 3/2, respectively. The hue of *P. australis* rhizomes changed from yellow (Y) to yellow-red (YR) from one- to six-years-old rhizomes and displayed distinct colours. The hue of the new rhizomes was YR. The value decreased from new to six-year-old rhizomes, indicating a darkening with ageing. Three- and four-year-old rhizomes, which had the same Munsell hue but different values, displayed highest chroma values (up to 8), while new rhizomes showed the lowest (chroma 1). All other age categories displayed chroma between 1 and 8.

Between 87% to 100% of the rhizomes attributed to a certain age class based on branching hierarchy were assigned to the same age class using colours (Table 1). New rhizomes having 100% throughout the growing season could be identified unmistakably by using the colour key, than all the other rhizome age groups. Also the oldest rhizome age group had comparatively higher percentage values (varying from 90% to 100%) throughout the sampling period. Generally, April and May sampling recorded comparatively higher percentage values in all age categories than the later months. However, all the age groups recorded percentage values more than 87. No specific pattern in percentages could be detected between the sampling dates and age groups.

Figure 4 was plotted by pooling all the data during the seven samplins, to confirm the relationship between the rhizome age based on branching hierarchy and rhizome age based on colour matching and showed a strong correlation ($r = +0.96$) between them. Hence, it was observed that the rhizomes of a specific age category could be assigned a distinct colour, being typical to that age category.

At each sampling, ρ_{rhiz} increased from new/one-year-old rhizomes to 6-year-old rhizome categories (Figure 5). After spring depletion, the young rhizomes strongly increased in ρ_{rhiz} compared with the older age categories. The correlation coefficient (r) between rhizome age and ρ_{rhiz} decreased from +0.99 in April to +0.45 in October. One-way ANOVA performed for each sampling date as well as for the entire data set (pooling values from all the sampling dates) always revealed significant effects of rhizome age on the ρ_{rhiz} (Table 2).

Discussion

Rhizome age identification and lifespan

The determination of rhizome age according to the position in the branching hierarchy has so far the most common practice for lack of more accurate alternatives. However, this is a tedious and time-consuming process, which has some well-known limitations. Errors can commonly arise from dating rhizome branches originating from the parent branch after a time interval of two years or more (Cizkova and Lukavska 1999). Cizkova and Lukavska (1999) successfully overcame this situation by using supplementary criteria, i.e., rhizome colour and condition of nodal sheaths, where colour was used as a comparative

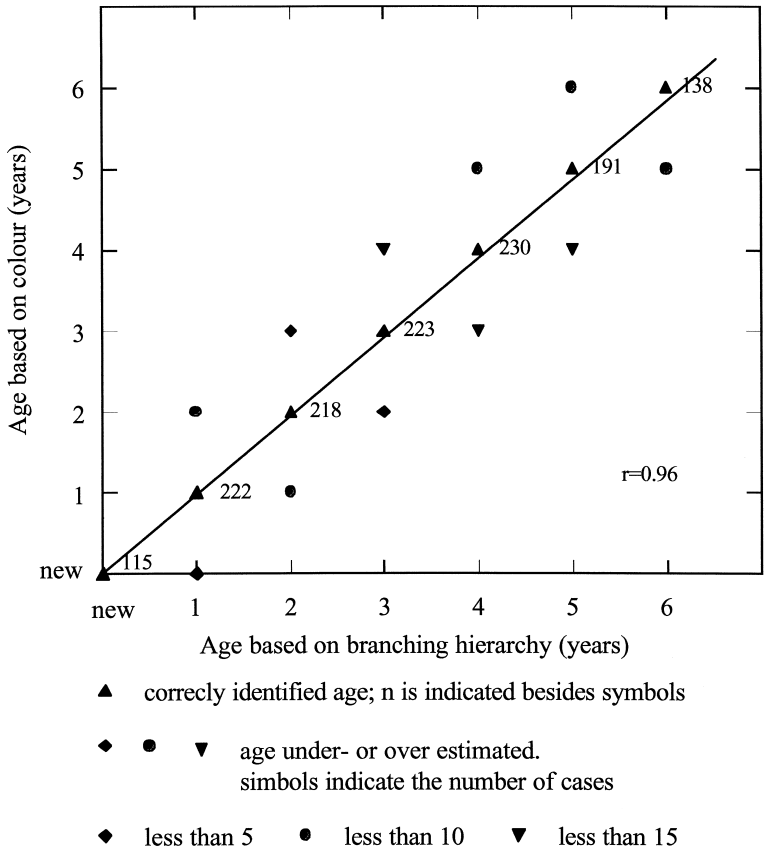

Figure 4. Correlation between the rhizome age based on the position in the branching hierarchy and the rhizome age based on colour, during all the samplings from April to October.

parameter. Even though in established populations rhizomes usually branch only once a year, two branching levels per year have been observed at the expanding edges of reed populations (Cizkova and Lukavska 1999; Haslam 1969). Further, if the shoots were harvested in the middle of the growing season, there would be shoots generated from the buds sprouted on one-year-old rhizomes, which bear the spring-formed shoots of the current growing season (Karunaratne et al. 2000). These factors complicate the identification of rhizome age by branching hierarchy. In such cases, the relative age of rhizomes can be easily and probably more accurately determined using a colour scheme. Another disadvantage of the rhizome age identification method by branching hierarchy is the need for large soil monoliths, which is, however, not always possible. High time and labour requirements usually associated with such work limit the number of samples being taken during a specific sampling date. Also, the disturbance to a larger area of the study site per sampling date limits the repeated sampling during a long-time period (e.g., throughout several consecutive growing seasons), if the selected stand is not large enough. Therefore, the present study was focused on developing a colour key to identify the age of *P. australis* rhizomes.

Using branching hierarchy to determine rhizome age, Fiala (1976) reported a lifespan of four years of *P. australis* rhizomes at Nesyt fishpond (South Moravia, Czech Republic) and Sakvicky fishpond (South Slovakia). Cizkova and Lukavska (1999) reported a rhizome life span of four, six and five years at Branna reed stand, Rozmberk fish pond (east shore) and Rozmberk fish pond (western

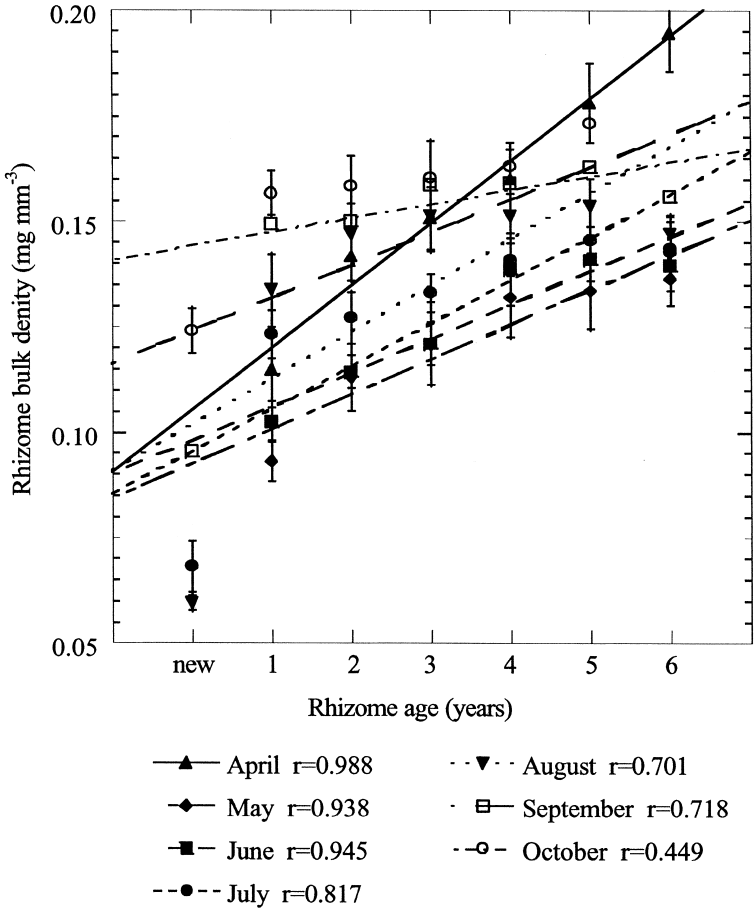

Figure 5. Correlations between the rhizome age and bulk density during sampling dates from April to October, 2000. Bars indicate standard error for means.

Table 2. One-way ANOVA testing for the differences of rhizome bulk density among the rhizomes of different age classes. There were six or seven age-classes depending on whether or not the new rhizomes included. For the values of bulk density of rhizomes, see Figure 5.

Sampling date	d.f.	*P*-value	*F*
28.04.00	5	<0.0001	9.3144
09.05.00	5	0.00034	3.9418
02.06.00	5	0.00041	5.0769
03.07.00	6	<0.0001	6.6509
01.08.00	6	0.00024	4.6908
07.09.00	6	<0.0001	9.3147
24.10.00	6	0.00010	2.4680
For pooled samples (excluding new rhizomes)	5	0.03727	2.4771

shore) (Trebon, Czech Republic), respectively. Using a method based on rhizome colour (as a comparative measurement), condition of leaf sheaths and roots, Klimes et al. (1999) defined the relative age of *P. australis* rhizomes as five and four years at Rozmberk fishpond (east shore) and Hruby Jesenik Mts (Czech Republic). Our data also indicate that the rhizomes of *P. australis* may live up to six years, but the lifespan may differ between localities.

Rhizome age and colour

It is well known that generally, rhizome colour become darker with increasing age. Even though few past studies (Cizkova and Lukavska 1999; Klimes et al. 1999) identified the usefulness of

colour in the determination of rhizome age, this method has not yet been subjected to a quantitative analysis. As far as the authors are aware the present study is the first of its kind to interpret rhizome colour using standard methods.

In colour research, selecting the shortest Euclidian distance between the sample object and the Munsell colour on the subspace is considered as the standard method. However, the present research is on the colour of living plant material and therefore the colour of a rhizome age category was defined as a range, consisting of two colours to achieve a more realistic representation. The proposed method of selecting colours as a range was further supported by the band-like deviations (width slightly increasing with increasing wavelength) of spectral reflectance that could be observed for all of the rhizome age categories and spectral reflectance distributions of corresponding Munsell colours almost completely falling within this band especially for one-, four-, five- and six-year-old rhizome categories.

Unlike length or weight, there is no scale for measuring the perceived colour. A certain colour is a matter of perception and subjective interpretation that needs a standard way of expression. Unlike the other more commonly used three-dimensional colour coordinate systems like, XYZ tristimulus values, CIELAB (L*a*b*), L*C*h*, CIELUV (L*u*v*) and Hunter Lab colour spaces in the present colour research (Minolta 1998), the Munsell colour charts are intended to be used for visual comparison with the specimen. The rhizome age identification method proposed in our study is especially useful in repeated measurement studies carried out in the same study site. Once a colour key is developed during the first sampling, using the spectral reflections, rhizome specimens can directly be compared visually making the identification faster and more efficient, in the subsequent samplings.

However, the rhizome colour scheme may be site-specific, depending on factors, such as pH, salinity, trophic level, redox state, substrate conditions of the site and genotype, remaining to be investigated further. Therefore, rhizome colour scheme should be verified for each stand investigated. Further, the rhizomes, very near to the soil surface should be excluded in identification of rhizome age by colour, as they have a tendency to get darker than the rest of the rhizome parts. This was observed especially in rhizomes older than three years or more, bearing a broken (near the soil surface) dead culm.

Therefore, the present study quantitatively showed that the rhizome colour could be used successfully and efficiently, as a tool in the determination of the relative age of *P. australis* rhizomes.

Rhizome age versus ρ_{rhiz}

The ρ_{rhiz} of rhizomes consistently increased with age at all seven samplings. The present observations correspond to the observations by Klimes et al. (1999) in two *P. australis* populations in Czech Republic during spring where glucose and starch concentration increased with rhizome age. Similar results have also been obtained with other species (Ralph et al. 1992; Klimesova and Klimes 1996). The strong positive correlations between age and ρ_{rhiz} observed during the early growing season and the poor correlations in the later growing season can be mainly attributed to the very low ρ_{rhiz} of newly formed rhizomes compared with older ones and also the rapid increase in ρ_{rhiz} of younger rhizomes compared with the older rhizomes. In addition, the six-year-old rhizomes did no longer confirm to the general pattern after the spring depletion of rhizome reserves. From June onwards, the ρ_{rhiz} of six-year-old rhizomes did not exceed that of five-year-old ones. In August and September, ρ_{rhiz} of six-year-old rhizomes was lower than that of three-year-old ones and only slightly higher than that of two-year-old ones. Six-year-old rhizome tissues did apparently no longer have the capacity to regain their pre-depleted state. This might be because they have reached the end of their lifespan and suggests again that six years is the maximum lifespan of *P. australis* rhizo mes at our site. In conclusion, this study has shown that colour can be used as a tool in the determination of the age of *P. australis* rhizomes.

Acknowledgements

We thank the Akigase Park office, Saitama, Japan for kindly allowing us to carry out the fieldwork within the Park premises. We are also thankful to K. Yutani and W. Sasaki for their help in

the fieldwork. We are grateful to H. Cizkova of the Institute of Botany of Czech Republic, for the valuable comments on our work. Moreover, we thank the editor and the anonymous referees for helpful comments that enabled us to improve the manuscript. For this study S.K. was financially supported by a research fellowship of the Japan Society for the Promotion of Science for young scientists while T.A. was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology.

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