Revised version for Applied Spectroscopy - Sept. 2009

Improved discrimination between monocotyledonous and dicotyledonous plants for weed control based on the blue-green region of UV-induced fluorescence spectra

Bernard Panneton[*], Serge Guillaume[†], Jean-Michel Roger[†], Guy Samson [‡]

October 2, 2009

Abstract - Precision weeding by spot spraying in real time requires sensors to discriminate between weeds and crop without contact. Among the optical based solutions, the UV induced fluorescence of the plants appears as a promising alternative. In a first paper, the feasibility of discriminating between corn hybrids, monocotyledonous and dicotyledonous weeds was demonstrated on the basis of the complete spectra. Some considerations about the different sources of fluorescence oriented the focus to the blue-green fluorescence part, ignoring the chlorophyll fluorescence that is inherently more variable in time . This paper investigates the potential of performing weed / crop discrimination on the basis of several large spectral bands in the BGF area. A partial least squares discriminant analysis (PLS-DA) was performed on a set of 1908 spectra of corn and weed plants, over 3 years and various growing conditions. The discrimination between monocotyledonous and dicotyledonous plants based on the blue-green fluorescence yielded robust models (classification error between 1.3 and 4.6% for between year validation). On the basis of the analysis of the PLS-DA model, two

[*]Horticultural R&D Centre, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, QC, Canada, J3B 3E6 - bernard.panneton@agr.gc.ca

[†]Cemagref, UMR ITAP, 34196 Montpellier, France

[‡]Département de chimie-biologie, Université du Québec à Trois-Rivières, Trois-Rivières, QC, Canada, G9A 5H7

large bands were chosen in the blue-green fluorescence zone (400-425 nm and 425-490 nm). A linear dicriminant analysis based on the signal from these two bands provided also very robust inter-year results (classification error from 1.5% to 5.2%). The same selection process was applied to discriminate between monocotyledonous weeds and maize but yielded no robust models (up to 50% inter year error). Further work will be required to solve this problem and provide a complete UV fluorescence based sensor for weed - maize discrimination.

KEYWORDS. fluorescence spectroscopy, monocotyledonous, dicotyledonous, discrimination, plant fluorescence

Introduction

Applications of plant-protection products targeted only to field areas requiring treatment (spot spraying) can substantially improve pesticide use by lowering the cost of plant protection and minimizing the impact on the environment. In weed control, potential savings from 9% up to 94% in herbicides have been reported[1]. However, implementation of spot spraying in commercial agriculture will require the development of sensing systems as components in automatic sprayer control systems[2].

For post emergence spraying of herbicides, the detection of weeds in the presence of the crop is necessary to perform spot treatment. Several optical techniques have been developed for this task based on different spectral characteristics and spatial features such as shape, texture and plant organization[3]. Approaches based on spectral characteristics of reflectance could be effective for some crop-weed systems under well controlled conditions. For example, broccoli and cabbage can be discriminated from weeds with a success rate of 100% and the rate was 88% for potatoes[4]. However, many have argued that the similarity between the spectral characteristics of weeds and crop species makes discrimination quite challenging when possible[3,5]. Furthermore, under field conditions, reflectance is affected by plant conditions and light fluctuation (sun angle or clouds) and the discrimination performance degrades[6]. Despite several decades of investigation, reflectance still faces important barriers for field implementation[7].

As spectral characteristics can be effectively used to isolate green plants in photographs, performing image analysis to extract spatial features can be performed for weed-crop discrimination. Based on shape features such as perimeter to surface ratio and roundness[8], discrimination between monocotyledonous and dicotyledonous plants was achieved with success rates between 75.0% and 98.6%. In an extensive study[9], 100 numerical features of

plant parts in image of green vegetation have been combined to 37 different classifiers to achieve plant classification in four groups (barley, rapeseed, broad-leaved weeds and grass weeds) with success rates over 95%. Key factors were the selection of the right numerical features (15 were selected) and the segmentation of the plant population into meaningful plant groups. More complicated approaches such as the deformable templates model[10] have been developed. Some of the major difficulties with the spatial features occur when plants are heterophyllous (different leaf shapes depending on development stage or environmental conditions) or when leaves of the same or different species overlap[11]. A robust and fast image processing approach has yet to be developed for the discrimination of weeds from crop under field conditions.

An interesting alternative to plant reflectance and image analysis could be the spectral analysis of UV-induced fluorescence that can be considered as a rich source of information still manageable and reliable. Potential applications of UV-induced fluorescence in plant monitoring have been reviewed[12,13] including species identification and detection of physiological disorders such as pathogens attacks and nutrient stresses. Dedicated instruments have also been developed. For example, the assessment of polyphenolic compounds in the leaf epidermis can be performed with the Dualex™ on attached leaves under field conditions[14]. This instrument has been shown to efficiently detect nitrogen deficiency in wheat crops[15]. Clearly, fluorescence spectroscopy is emerging as a monitoring tool in agriculture. Other approaches based on fluorescence such as fluorescence imaging[16] or chlorophyll fluorescence induction[17] have also been investigated but would be difficult or impossible to implement under field conditions for crop-weed discrimination in real time.

Under UV-excitation, plants can emit a wide fluorescence spectrum ranging from about 400 to 800 nm. This spectrum is the sum of two distinct types of fluorescence, the blue-

green fluorescence (BGF) characterized by a peak around 440 nm ($F440$) and a shoulder around 530 nm ($F530$), and the chlorophyll-a fluorescence (ChlF) in the red/far-red region with its two characteristic peaks at about 685 nm ($F685$) and 735 nm ($F735$). Most of the BGF detected from intact leaves is emitted by ferulic acid (a phenolic compound) bound to the cell walls[18]. The relative intensities of BGF and ChlF are determined by leaf intrinsic properties, notably ferulic acid and chlorophyll-a concentrations and also UV-transmittance of leaf epidermis that is influenced by UV-absorbing metabolites[19]. These leaf properties vary according to plant species, leaf development and environmental conditions. Further, the relative intensities of BGF and ChlF depend also on the excitation wavelength owing to the different absorbance spectra of ferulic acid and chlorophyll-a as well as of other UV absorbing compounds[20].

Image analysis has revealed a significant spatial heterogeneity of fluorescence emitted from the leaf surface. Most of ChlF originate from leaf blade whereas intensity of BGF is higher in tissues such as leaf veins and leaf epidermis (seen in cross-section) where chlorophyll concentration is low. In monocotyledons, the basal development of leaves results in a longitudinal gradient where the intensities of both BGF and ChlF decreases from the base to the apex of the leaves[21,22].

Since several intrinsic leaf properties determine plant fluorescence, the characteristics of its emission spectrum can be considered as a distinct and meaningful signature that can be used notably for plant discrimination. In one of the early studies on plant fluorosensing[23], discrimination between four plant groups (conifers, hardwoods, herbaceous monocotyledons and dicotyledons) was based on the ratio of blue fluorescence squared to red fluorescence intensities ($F440^2/F685$) induced by a nitrogen laser at 337 nm. Compared to dicots, monocots and more precisely members of the *Poaceae* have several times higher content of ferulic

acid[18]. As a result, monocot leaves emit more BGF than dicot leaves, resulting in higher $F440/F685$ ratio. Moreover, it has been observed that the $F685/F735$ ratio could discriminate four plant species: peas, barley, clover and Shepherd's purse (*Capsella bursa-pastoris* L. Med.)[24]. However, since the relative intensities of $F685$ and $F735$ are mostly related to leaf chlorophyll concentrations[25], the significance of this fluorescence ratio for plant discrimination may be limited.

More recently, Panneton and his collaborators have been able to successfully discriminate plants from three groups relevant for weed control, corresponding to four corn hybrids, four monocot weeds and four dicot weeds. To this end, a multivariate analysis has been used to first reduce the relevant information contained in each spectrum to scores calculated from five principal components. Then, a linear discriminant analysis has been applied to classify these scores on a species/hybrids basis with a success rate reaching 91.8%[26]. The spectra used in these studies have been collected from different leaf positions and from leaf of different developmental stages to ensure some robustness of the multivariate models. Although encouraging, these models have not been applied to spectra obtained from plants grown under different conditions or exposed to different conditions during fluorescence measurements. Therefore, robustness of the models remains an important issue in their transfer to field conditions.

Considerations about the distinct natures of BGF and ChlF could help to improve the discrimination between monocotyledonous and dicotyledonous plants for weed control under diverse conditions. In leaves of all species, ChlF is emitted exclusively by chlorophyll-a and shows always the two characteristics peaks near 685 nm and 735 nm[12]. Their relative intensities depend on the wavelength of excitation and on chlorophyll concentrations, factors that have low potential for plant discrimination. Also, ChlF directly depends on photosynthetic

efficiencies that can vary rapidly depending on ambient conditions[27]. Finally, light quality during growth and more specifically its UV content can induce the synthesis of polyphenolic compounds in leaf epidermis. These compounds are strong absorbers of UV radiation and decrease the UV-excitation of chlorophyll molecules located below in mesophyll cells[19]. Overall, the rapid variations of ChlF due to ambient light intensity and variations caused notably by growth light quality can decrease the efficiency of the entire UV-induced fluorescence spectra for plant discrimination.

Contrary to ChlF, BGF remains constant despite rapid variations of ambient light intensities[28] that can occur during fluorescence measurements in the field[29]. It can however decrease by 1% to 5% when leaf temperature increases by 1°C[28,30]. On the other hand, the contributions of different molecules besides ferulic acid to BGF emission[12] can explain the significant species-dependent variations of the BGF excitation and emission spectra reported in a survey performed for 35 species grouped under seven different life forms[20].

Taken together, the above considerations led us to hypothesize that the analysis the BGF region of the UV-induced fluorescence spectrum can yield a more robust fingerprint for species compared to the full fluorescence spectrum that includes both BGF and ChlF. Therefore, the objectives of the work reported here were first to demonstrate the higher ability of BGF alone, compared to the full fluorescence spectrum (BGF and ChlF), to first discriminate monocotyledonous from dicotyledonous plants, and then to discriminate monocotyledonous from maize hybrids. The discriminating abilities of BGF were based either on its detailed spectral characteristics or on a limited set of large wavebands defined on the basis of a partial least square discriminant analysis (PLS-DA). To ensure robustness of the fluorescence discriminating models, plants have been grown either under natural sunlight in a greenhouse or in growth chamber under metal halide lamps. Also, fluorescence measurements have been

made under different ambient light at two growth stages and at two contrasting locations on the leaves. These results could help to define a sensor based on the characteristics of a UV-induced fluorescence for plant species discrimination within the context of weed control.

Materials and Methods

Plant material and experiments

The experimental program spanned over three years (2005, 2006 and 2007). Each year, the same species were used. These were in three groups (see Table 1 for species names): four *Zea Mays* (L.) hybrids, four annual monocotyledonous grasses and four annual dicotyledonous weeds. Corn is a monocotyledonous plant. In 2005 and 2006, plants were grown in a growth chamber and in a greenhouse in 2007. In 2005 and 2006, light was provided by 1000 W metal halide lamps at a distance of approximately one meter from the pots giving about 600 W/m^2 or 500 μmol/m^2/s. In the greenhouse, high pressure sodium light were used to supplement sunlight. For all years, the photoperiod was 16 hours of light and 8 hours of darkness. In the growth chamber, the temperature was set to 20°C during the day and 12°C at night, with a plateau of one hour at 16°C between each changes of temperature. In the greenhouse, the maximum temperature was set to 24°C and night temperature was maintained above 12°C. Plants have grown in 1.07 L pots (127 mm dia.) of soil-less mix (Promix BX, Premier Horticulture, Quebec, Canada). Nutrients (20-12-20 at 95 g/L) were dissolved in tap water and was applied at every watering. Care was taken to avoid systematic temperature and lighting gradients by moving around pots every other day.

For each pot, the date of emergence was recorded and fluorescence measurements were made between 15 and 20 days after emergence (stage 1) and again on the same plants,

between 25 and 30 days after emergence (stage 2). Fluorescence was measured on the uppermost fully developed leaf. At stages 1 and 2, two measurements were performed on each leaf. For the dicotyledons, one spectrum was read of the main leaf vein and another one in the leaf blade. For the monocotyledons, one measurement was performed on a point near the base of the leaf (lower 25% of the leaf blade) and another one near its apex (top 25% of the leaf blade). In 2005 and 2007, measurements were performed in a greenhouse under natural daylight. Plants were placed in the greenhouse one hour before measurements. In 2006, measurements were performed under metal halide lamps at 500 μmol/m^2/s and 20°C. In 2006, the ambient conditions were stable and selected to be close to the mean conditions obtained in 2005 and 2007 in the greenhouse environment (averages of 440 μmol/m^2/s and 22°C). Each year, the experiment was repeated in time at three occasions (3 blocks of data). In each block, there were 8, 4 and 4 specimens of each species/hybrid for 2005, 2006 and 2007 respectively. Therefore, the experiments were planned to provide a total of 2304 spectra (3 years, 3 blocks, 3 plant groups, 4 hybrid/species per group, 4 or 8 replicates per hybrids/species and 4 readings for each). In the end, some data were rejected for various reasons (growth problems, instrumentation problems...) yielding a validated data set made of 1908 spectra.

Fluorescence measurements

Plant fluorescence was induced by a xenon flash lamp (Spectra-physics Series Q Housing 60000 with a 5J Xe pulsed arc lamp) controlled by an Oriel 68826 power supply (9 μs pulse width). The flash output was coupled to a fiber optic bundle (Oriel 77578) using a condensing lens assembly (Oriel 60076) and a bandpass filter centered at 327 nm (20 nm FWHM). The induced fluorescence was collected by another fiber-optic bundle (Oriel 77532) and transported to the spectrograph (Oriel MS125 1/8m). Using a length gauge,

both fiber optics were positioned 5 mm above the leaf and pointed to the same spot on the leaf, 2.3 mm in diameter. The leaf blade was positioned perpendicular to the probe as judged by the operator. The spectrograph was modified by the insertion of a high-pass filter at the input port (400 nm: 5% at 388 nm and 80% at 405 nm) to cut-off second-order effects. An ICCD detector (Andor, DH 712-18F/03, 5ns, Phosphore P43) was coupled to the spectrograph to record the spectrum in the range from 400 to 760 nm (378 pixels or wavebands). Fluorescence signals were acquired using the same technique as Norikane and Kuruta[31]. Under ambient light (sunlight in 2005 and 2007 or lamps in 2006) and without the UV excitation, 11 spectra were acquired at 10 Hz and averaged. Then, 11 spectra were acquired under UV flash excitation (10 Hz) and averaged . The difference between the two resulting spectra is the pure induced fluorescence signal.

Data processing and analysis

The fluorescence spectra were smoothed using the Savitzky-Golay technique[32] with a third order polynomial and a 21 channel wide window. Pre-processing involved division of the whole spectra by the integral in the 570-620 nm band as defined previously[33].

Further processing was performed in Matlab®using custom scripts and the PLS_Toolbox[32]. Models for discriminating monocotyledonous from dicotyledonous plants based on complete normalized spectra were constructed using the partial least square discriminant analysis (PLS-DA,[34]) as implemented in the PLS_Toolbox. The number of latent variables in the models was selected from the analysis of the cross-validation error as a function of the number of latent variables. Cross-validation was of the venetian-blind type[32]. Models calibrated with data from one year have been applied to data from the other two years and prediction errors were computed to evaluate model robustness.

Plots of the regression coefficient and of the variable importance in projection (VIP)[32] were used to identify potential spectral bands for a simplified discrimination model based on few wavebands. In this case, a linear discriminant analysis (LDA)[35] was performed to define a data space for discrimination between the two classes. Here again, a discriminant space calibrated with data from one year was applied to data from the other 2 years to obtain cross-validation and prediction errors. Prediction was based on the minimum of the Mahalanobis distances[35] to each class.

Results and Discussion

Normalized fluorescence spectra

For each year, the amplitudes of the normalized spectra in the blue-green region (400 to 625 nm) followed the same order with respect to plant groups (Fig. 1). Maize had the largest amplitude followed by monocotyledonous and then by dicotyledonous weeds. In the red fluorescence band (625 nm and above, this is the chlorophyll fluorescence or ChlF), there was no consistent order from year to year. However, when data from different years were compared, the amplitude of the BGF for monocotyledonous weeds in 2006 was almost as high as the ones of maize in 2005 and 2007. These observations suggest that the good separation on a yearly basis between mean spectra in the blue-green fluorescence (BGF) explains the high success rate in classification based on full spectra that was obtained previously[26,33]. These observations further suggest that a classification model calibrated on a data set may not be robust when applied to new data acquired under different ambient conditions.

It is noteworthy that BGF was generally highest in 2006 although plants from both 2005 and 2006 were grown in a growth chamber. For each of the three plant groups, 2006 was also

the year where ChlF was lower while it was higher in 2007. These differences could be related to the fact that in 2005 and 2007, measurements were performed under natural light in the greenhouse whereas in 2006, the measurements were performed under artificial lighting. In the PAR region (400 to 700 nm), most of the photons from metal halide lamps are concentrated in the 560-640 nm range (www.sunmastergrowlamps.com/SunmNeutral.html; June 15, 2009) corresponding to the lower absorbance region of the chlorophylls. It is therefore possible that compared to natural sunlight, light provided by the lamps was less absorbed, causing a lower excitation pressure on the photosynthetic apparatus. In consequence, a lower proportion of closed photosystem II would result in lower emission of ChlF[27], and then a relative increase of BGF.

Monocot-Dicot discrimination based of full spectra

Yearly PLS-DA models based on the full normalized spectra (BGF + ChlF) were constructed to discriminate monocots from dicots. Results showed cross-validation errors of 1.7%, 1.7% and 3.7% for years 2005, 2006 and 2007 respectively (left side of Table 2). When these models were applied to data from other years, the prediction error was between 4.8% and 13.4% (Table 2). Such difference between the cross-validation and the prediction errors indicated that some year to year variations were present. To gain some insight about the origin of this variation, boxplots of normalized spectra in the blue fluorescence (BF : 420-450 nm range), red fluorescence (RF : 675-695 nm) and far-red fluorescence (FRF : 725-735 nm) bands were prepared for one species for each of the three plant groups (Fig. 2). In the blue fluorescence, the relative position of each plant did not change and data from 2005 and 2007 were very similar while there was an increase in 2006 and that was more pronounced for the monocot plant (DI). In the ChlF area (both RF and FRF), the pattern between species varied from

year to year although the maize consistently showed lower ChlF. These observations provided an indication that working solely with the BGF part of the spectra might offer better year to year robustness.

Monocot-Dicot discrimination based of BGF spectra

Using BGF alone, yearly PLS-DA models resulted in cross-validation errors of 1.2%, 1.9% and 3.7% for years 2005, 2006 and 2007 respectively (right side of Table 2). These errors were almost equal to the ones obtained with models based on the full spectra. On the other hand, the prediction errors calculated from data sets of different years were significantly reduced and ranged between 1.3% and 4.6%. These prediction errors were about four times lower that the one calculated from the full spectrum. This showed that discrimination based of BGF alone was more robust than discrimination based on the full fluorescence spectra.

It is interesting to note that for the PLS-DA model based only on BGF, there were only 2 latent variables retained. For 2 to 5 latent variables, both the calibration and the cross-validation errors were constant and past 5 latent variables, the cross-validation error started to increase (Fig. 3). Variable importance in projection (VIP) plot showed that data in the spectral range from 400 nm to about 490 nm contributed more to the discrimination model (VIP>1). In this range, the regression coefficients were positive from 400 nm to 425 nm and negative from 425 nm to 490 nm (Fig. 4). These two results suggested that a simple classification model based on two bands (400-425 nm and 425-490 nm) could be sufficient to separate dicots from monocots.

Monocot-dicot discrimination based on selected spectral bands of BGF

Boxplots of mean normalized fluorescence in two bands (400-425 nm and 425-490 nm) showed clear patterns that were stable from year to year (Fig. 5). In 2007, the variability in the data for the monocots, in both bands, was greater with more extreme values.

The cross-validation errors for LDA models based on the 400-425 nm and 425-490 nm bands were 1.6%, 1.7% and 5.0% for years 2005, 2006 and 2007 respectively (Table 3). These errors were comparable to the one for the PLS-DA models based on the BGF except for 2007 where the error was greater with the LDA model. With the LDA models, the prediction error was from 1.5% to 5.2%. Again, errors associated with year 2007 were systematically higher. For 2005 and 2006, the differences between the cross-validation and the prediction errors were small.

Prediction errors associated with each of the species or hybrids were calculated to verify if the prediction error was uniformly distributed across species/hybrids (Fig. 6). One of the species (AA) and three hybrids (E, P and S) were always well classified. Prediction errors associated with CB and DI were generally higher and overall, the error was higher with monocots (except for dicot CB). Boxplots of the value on the discriminant axis for each species and hybrids (Fig. 7) showed that there was a more significant confusion between CB and DI and that for monocots, there were more outliers (circles on the figure). As a more even distribution of the error across species/hybrids was desirable, a LDA model based on three bands (400-425; 425-490; 500-550) was evaluated. Overall, there was no gain in accuracy and the distribution of errors was not improved (data not shown) indicating than increasing model complexity in the BGF range did not improve classification potential.

Monocot-Maize discrimination based of full spectra

Given the success of the approach for discriminating between monocots and dicots, it was tested for the discrimination between monocotyledonous weeds and maize. PLS-DA models for the monocot-maize discrimination required more latent variables than for monocot-dicot discrimination. For the models based on the full spectra, there were 3, 4 and 3 latent variables for the 2005, 2006 and 2007 calibration data sets respectively while for the models based on the BGF spectra, there were 4, 3 and 4 latent variables. The cross-validation error ranged from 4.8% to 8.3% and from 4.0% to 13.6% for models based on the full and BGF spectra respectively (Table 4). In both cases, the prediction error was higher ranging from 11.0% to 50.0%. For a two-class model, a prediction error of 50% is equivalent to pure chance. The cross-validation results indicated that the discrimination between maize and monocotyledonous weeds can be achieved and that the model based on the full spectra was better than the model based on the BGF spectra. However, the lack of robustness indicated by the high prediction errors will have to be addressed. Given these results, the band selection process applied previously was not tested for the discrimination between maize and monocots.

Conclusion

In the context of weed control in maize production, this work investigated whether it is possible to discriminate between monocotyledonous and dicotyledonous plants based on UV-induced fluorescence observed in the BGF range. The main conclusion is that this was feasible using only two fluorescence bands at 400-425 nm and 425-490 nm with a classification success rate better than 94.8%. Moreover, attention has been paid to robustness. The

experiments have been carried out with a multiyear data set corresponding to various growth and measurement conditions and with measurements performed on plants at different growth stages and varying the location of measurement on the leaves. Also, the measurement bands were made as large as possible in order to increase the signal to noise ratio. Therefore, it is further concluded that the discrimination strategy should be robust with respect to ambient conditions and growth stage.

For weed control in agriculture, the final goal is not only monocot and dicot discrimination but also to isolate the crop from the weeds. The approach based on PLS-DA applied to detailed spectra was tested for the discrimination of maize and monocotyledonous weeds. Based on cross-validation within data sets where growth and measurement conditions were fixed, the results showed that discrimination based on the full fluorescence spectra (ChlF and BGF) can be performed with a success rate better that 91.7%. Strategies for improving robustness for maize-monocot discrimination have to be developed.

Acknowledgements

The excellent technical support of Mr G. St-Laurent and Mrs M. Piché is hereby acknowledged. This work was financially supported by Agriculture and Agri-Food Canada and by a Natural Sciences and Engineering Research Council of Canada Discovery Grant.

References

1. B. Panneton, L. Longchamps, R. Thériault, and G.D. Leroux. Fluorescence spectroscopy of vegetation for weed-crop discrimination. In *ASABE paper 061063*, 2006.

2. P.C.H. Miller. Patch spraying: future role of electronics in limiting pesticide use. *Pest Management Science*, 59:566–574, 2003.

3. R. B. Brown and S. D. Noble. Site-specific weed management: sensing requirements - what do we need to see? *Weed Science*, 53:252–258, 2005.

4. E. Hahn and A. Y. Muir. Spectral sensing for crops and weed discrimination. *Acta Horticulturae*, 372:179–186, 1994.

5. J. V. Stafford. Implementing precision agriculture in the 21th century. *Journal of Agricultural Engineering Research*, 76:267–275, 2000.

6. P. Lutman and N. H. Perry. Methods of weed patch detection in cereal crops. In *Brighton Crop Protection Conference - Weeds*, pages 627–634, Farnham, UK, 1999. British Crop Protection Council, British Crop Protection Council.

7. D. Slaughter, D. Giles, and D. Downey. Autonomous robotic weed control systems: A review. *Computers and Electronics in Agriculture*, 61:63–78, 2008.

8. I. Schuster, H. Nordmeyer, and T. Rath. Comparison of vision-based and manual weed mapping in sugar beet. *Biosystems Engineering*, 98:17–25, 2007.

9. M. Weisa and R. Gerhards. Feature extraction for the identification of weed species in digital images for the purpose of site-specific weed control. In *Precision agriculture*

'07, Papers presented at the 6th European Conference on Precision Agriculture, pages 537–544, Skiathos, Greece, June, 3-6 2007.

10. A. Manh, G. Rabatel, L. Assemat, and M. Aldon. Ae-automation and emerging technologies weed leaf image segmentation by deformable templates. *Journal of Agricultural Engineering Research,*, 80:139–146, 2001.

11. D. R. Shaw. Remote sensing and site-specific weed management. *Frontier in Ecology and Environment*, 3:526–532, 2005.

12. Z. G. Cerovic, G. Samson, F. Morales, N. Tremblay, and I. Moya. Ultraviolet-induced fluorescence for plant monitoring: present state and prospects. *Agronomie*, 19:543–578, 1999.

13. *Proceedings of the 2nd International Workshop on Remote Sensing of Vegetation Fluorescence.*, volume ESA WPP-242, Canadian Space Agency, St-Hubert, Qc, Nov, 17-19 2004. European Space Agency, ESA Publications Division.

14. Y. Goulas, Z. G. Cerovic, and I. Cartelat, A.and Moya. Dualex: A new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. *Applied Optics*, 43:4488–4496, 2004.

15. A. Cartelat, Z.G. Cerovic, Y. Goulas, S. Meyer, C. Lelarge, J.-L. Prioul, A. Barbottin, M.-H. Jeuffroy, P. Gate, G. Agati, and I. Moya. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (triticum aestivum l.). *Field Crops Research*, 91:35–49, 2005.

16. C. Buschmann, G. Langsdorf, and H. K. Lichtenthaler. Imaging of the blue, green and red fluorescence emission of plants: An overview. *Photosynthetica*, 38:483–491, 2000.

17. M. Keränen, E. M. Aro, and E. Tyystjärvi. Automatic plant identification with chlorophyll fluorescence fingerprinting. *Precision Agriculture*, 4:53–67, 2003.

18. H.K. Lichtenthaler and J. Schweiger. Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants. *Journal of plant physiology*, 152: 272–282, 1998.

19. W. Bilger, M. Veit, L. Schreiber, and U. Schreiber. Measurement of leaf epidermal transmittance of uv radiation by chlorophyll fluorescence. *Physiologia Plantarum*, 101: 754–763, 1997.

20. G. A. Johnson, S. V. Mantha, and T. A. Day. A spectrofluorometric survey of uv-induced blue-green fluorescence in foliage of 35 species. *Journal of Plant Physiology*, 156:242–252, 2000.

21. G. Langsdorf, C. Buschmann, M. Sowinska, F. Babani, M. Mokry, F. Timmermann, and Lichtenthaler H.K. Multicolour fluorescence imaging of sugar beet leaves with different nitrogen status by flash lamp uv-excitation. *Photosynthetica*, 38:539–551, 2000.

22. S. Meyer, A. Cartelat, I. Moya, and Z.G. Cerovic. Uv-induced blue-green and far-red fluorescence along wheat leaves: a potential signature of leaf ageing. *Journal of Experimental Botany*, 54:757–769, 2003.

23. E. W. Chappelle, F.M. Wood, W.W. Newcomb, and J.E. McMurtrey III. Laser-induced fluorescence of green plants. 3: Lif spectral signature of five major plant types.". *Applied Optics*, 24:74–80, 1985.

24. P. J. Hilton. Laser-induced fluorescence for discrimination of crops and weeds. In *Proceedings of the SPIE*, volume 4124, pages 223–231, 2000.

25. A. A. Gitelson, C. Buschmann, and H. K. Lichtenthaler. The the chlorophyll fluorescence ratio f735/f700 as an accurate measure of the chlorophyll content in plants. *Remote sensing of the environment*, 69:296–302, 1999.

26. L. Longchamps, B. Panneton, G. Samson, G. D. Leroux, and R. Thériault. Discrimination of corn, grasses and dicot weeds by their uv-induced fluorescence spectral signature. *Precision Agriculture*, 2009.

27. K. Maxwell and G. Johnson. Chlorophyll fluorescence - a practical guide. *Journal of experimental botany*, 51:659–668, 2000.

28. G. Bongi, A. Palliotti, P. Rocchi, I. Moya, and Y. Goulas. Spectral characteristics and possible topological assignment of blue green fluorescence excited by uv laser on leaves of unrelated species. *Remote Sensing of the Environment*, 47:55–64, 1994.

29. F. Stober and H.K. Lichtenthaler. (1993) studies on the constancy of the blue and green fluorescence yield during the chlorophyll fluorescence induction kinetics (kautsky effect). *Radiation and Environmental Biophysics*, 32:357–365, 1993.

30. F. Morales, Z.G. Cerovic, and I. Moya. Time-resolved blue-green fluorescence of sugar beet leaves. temperature-induced changes and consequences for the potential use of blue-green fluorescence as a signature for remote sensing of plants. *Australian Journal of Plant Physiology*, 25:325–334, 1998.

31. J.H. Norikane and K. Kuruta. Water stress detection by monitoring fluorescence of plants under ambient light. *Transactions of the ASAE*, 44:1915–1922, 2001.

32. B.M. Wise, N. B. Gallagher, R. Bro, J. M. Shaver, W. Windig, and R.S. Koch.

PLS_Toolbox 4.0 - Reference Manual for use with Matlab. Eigenvector Research Inc., 2006.

33. B. Panneton, J. M.l Roger, S. Guillaume, and L. Longchamps. Effects of preprocessing of ultraviolet-induced fluorescence spectra in plant fingerprinting applications. *Applied Spectroscopy*, 62:747–752, 2008.

34. M. Barker and W. Rayens. Partial least square for discrimination. *Journal of Chemometrics*, 17:166–173, 2003.

35. G. Saporta. *Probabilités, Analyse des données et statistiques*. Editions Technip, 1990.

Table 1: List of weed species and corn hybrids.

Plant Group	Species or corn hybrids	Code
Corn hybrids	Elite 60T05	E
	Monsanto DKC 26-78	M
	Pioneer 39Y85 (RR)	P
	Syngenta N2555 (*Bt*, LL)	S
Monocotyledoneous weeds	*Digitaria ischaemum* (Schreb.) I	DI
	Echinochloa crus-galli (L.) Beauv.	EC
	Panicum capillare (L.)	PC
	Setaria glauca (L.) Beauv.	SG
Dicotyledoneous weeds	*Ambrosia artemisiifolia* (L.)	AA
	Amaranthus retroflexus (L.)	AR
	Chenopodium album (L.)	CA
	Capsella bursa-pastoris (L.) Med.	CB

Table 2: Cross-validation and prediction errors for the PLSDA model on full and BGF spectra for monocot-dicot discrimination. Row headings identify the year of the calibration data set. Column headings give the year used to test the model. Terms on the diagonal are cross-validation errors.

	Full spectra				BGFspectra		
	2005	2006	2007		2005	2006	2007
2005	1.7%	4.8%	9.2%		1.2%	1.7%	3.9%
2006	5.3%	1.7%	11.0%		1.3%	1.9%	4.6%
2007	13.4%	6.4%	3.7%		1.8%	2.2%	3.7%

Table 3: Cross-validation and prediction errors for the LDA model based on two bands (400-425 nm and 425-490 nm) for monocot-dicot discrimination. Row headings identify the year of the calibration data set. Column headings give the year used to test the model. Terms on the diagonal are cross-validation errors.

	Two bands		
	2005	2006	2007
2005	1.6%	2.1%	5.2%
2006	1.5%	1.7%	4.3%
2007	2.0%	2.6%	5.0%

Table 4: Cross-validation and prediction errors for the PLSDA model on full and BGF spectra for maize-monocot discrimination. Row headings identify the year of the calibration data set. Column headings give the year used to test the model. Terms on the diagonal are cross-validation errors.

	Full spectra				BGF spectra		
	2005	2006	2007		2005	2006	2007
2005	4.8%	32.4%	48.3%		7.9%	42.6%	50.0%
2006	11.0%	4.9%	39.2%		28.7%	4.0%	36.1%
2007	41.0%	44.5%	8.3%		45.7%	42.7%	13.6%

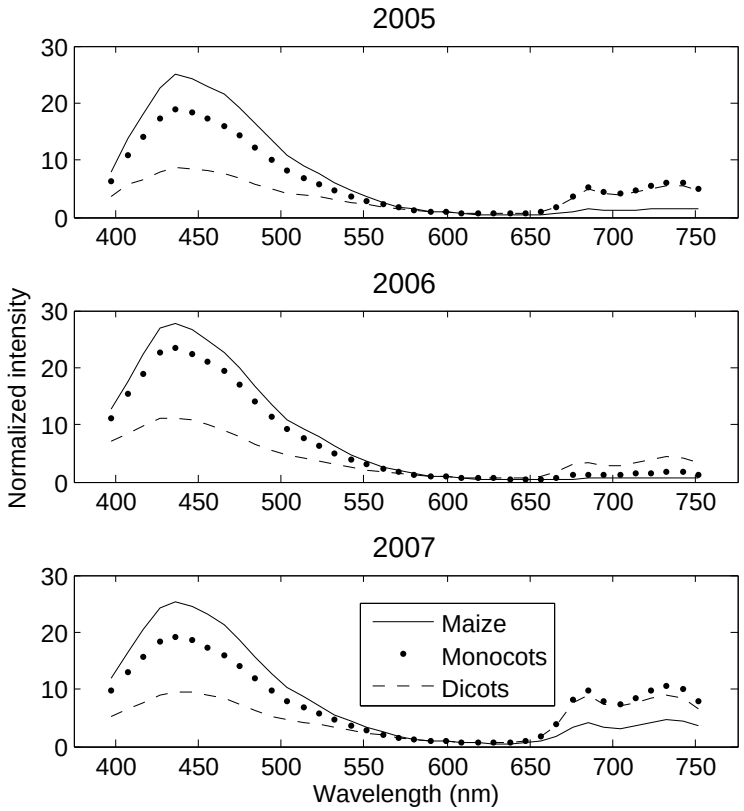

Figure 1: Mean normalized spectra. Averaged over year and type.

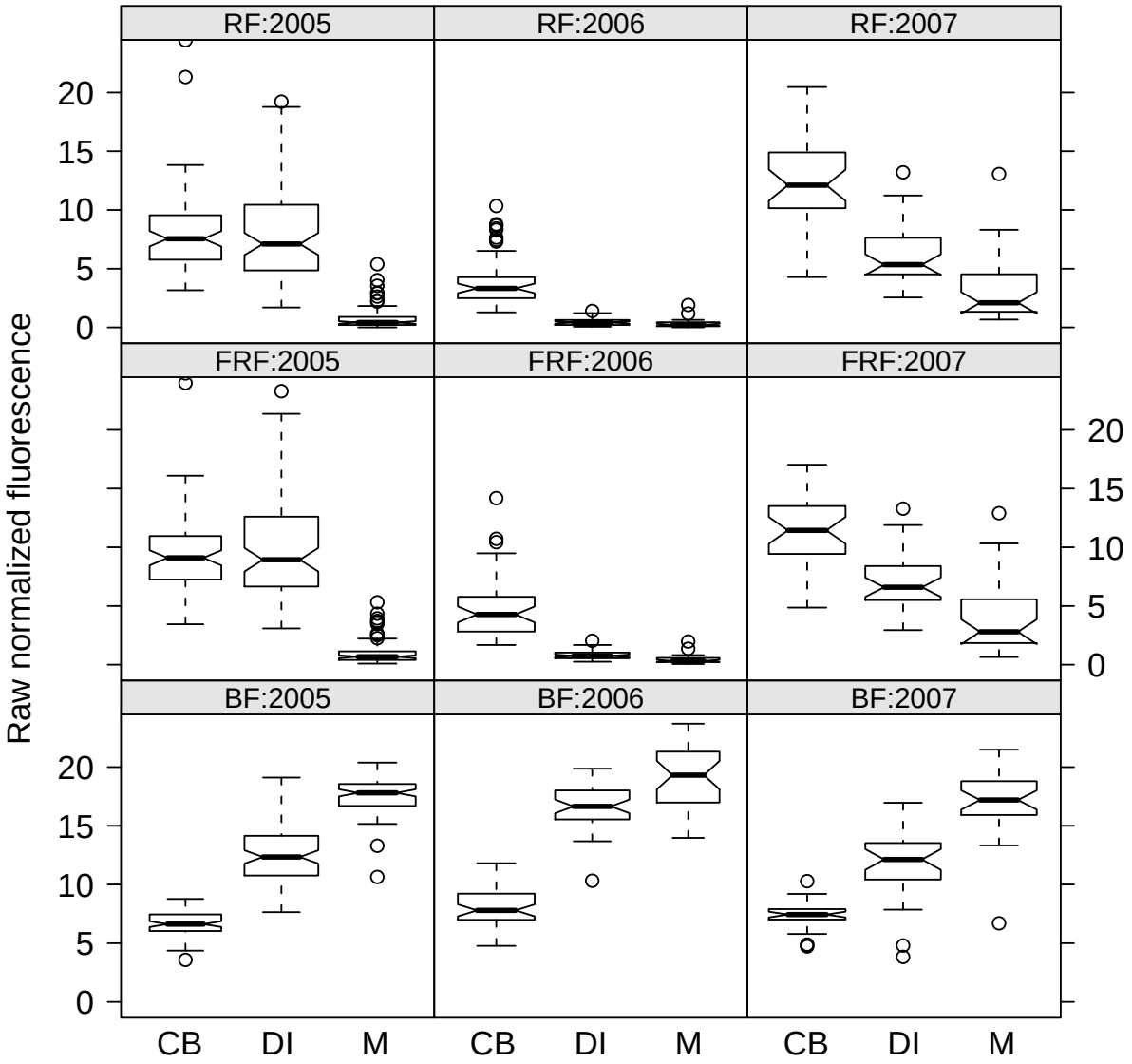

Figure 2: Boxplots of blue fluorescence (BF: 420-450 nm), red fluorescence (RF: 675-695 nm) and far-red fluorescence (FRF: 725-745 nm) per year for CB, DI and M. Normalized data. FRF and RF were amplified by a factor of 2 to improve the readability of the graphs. . The abbreviations labeling the horizontal axis refer to the code given in Table 1

.

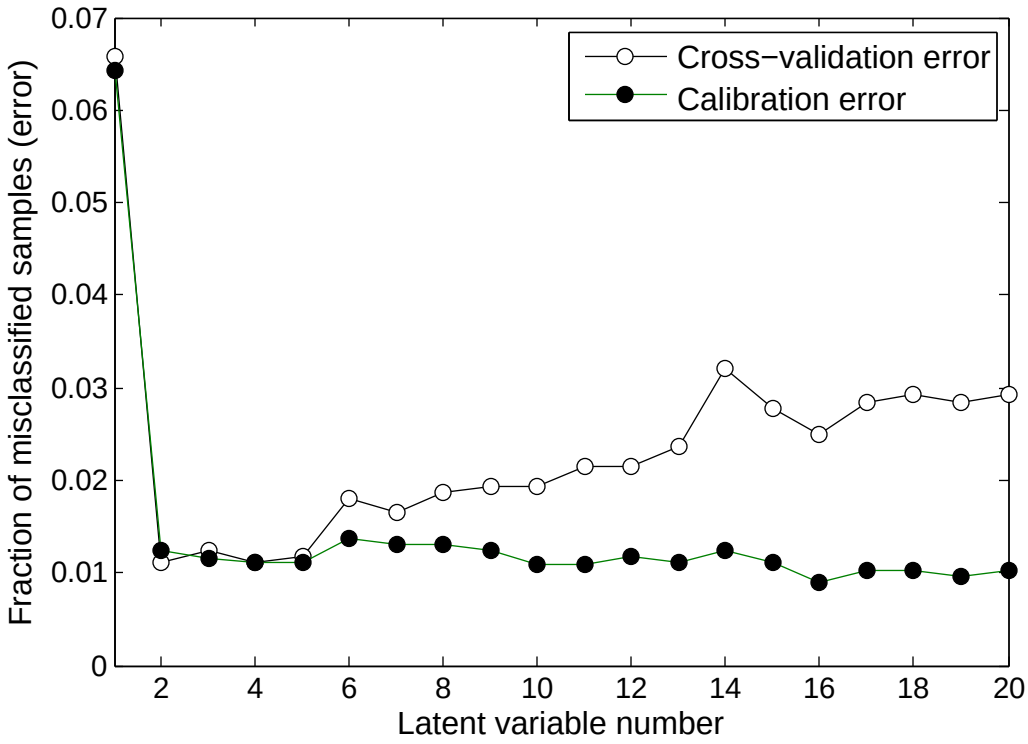

Figure 3: Calibration/validation error versus number of latent variables for blue-green fluorescence spectra.

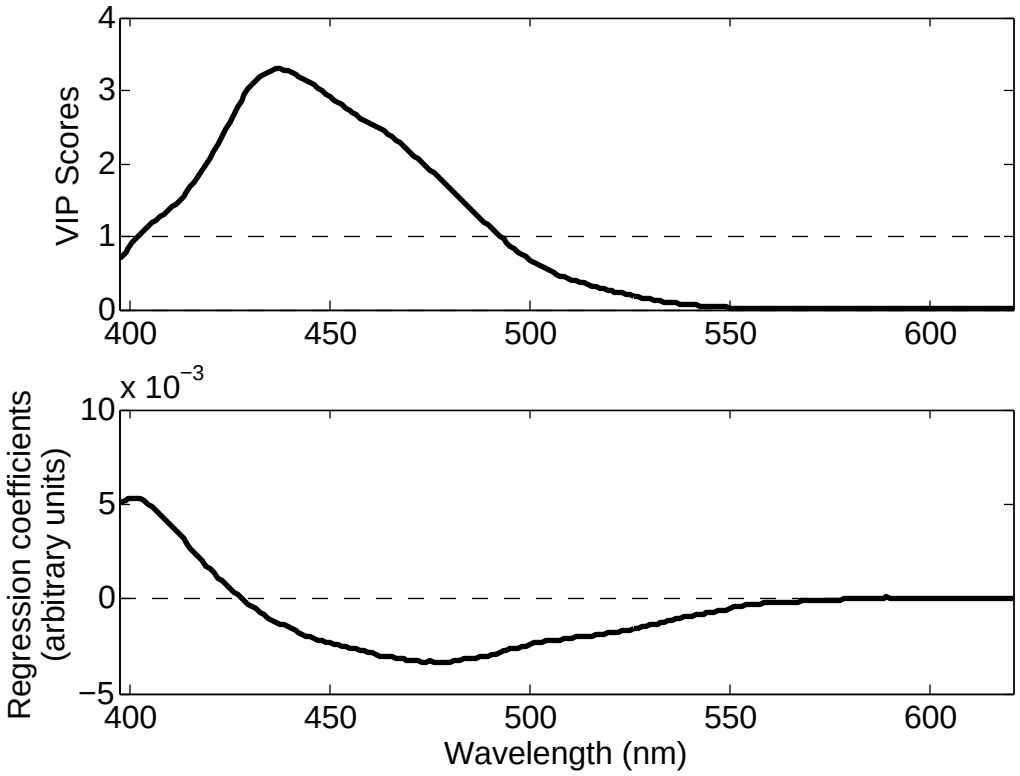

Figure 4: Variable importance in projection (VIP) and regression coefficients from PLSDA analysis on blue-green fluorescence spectra.

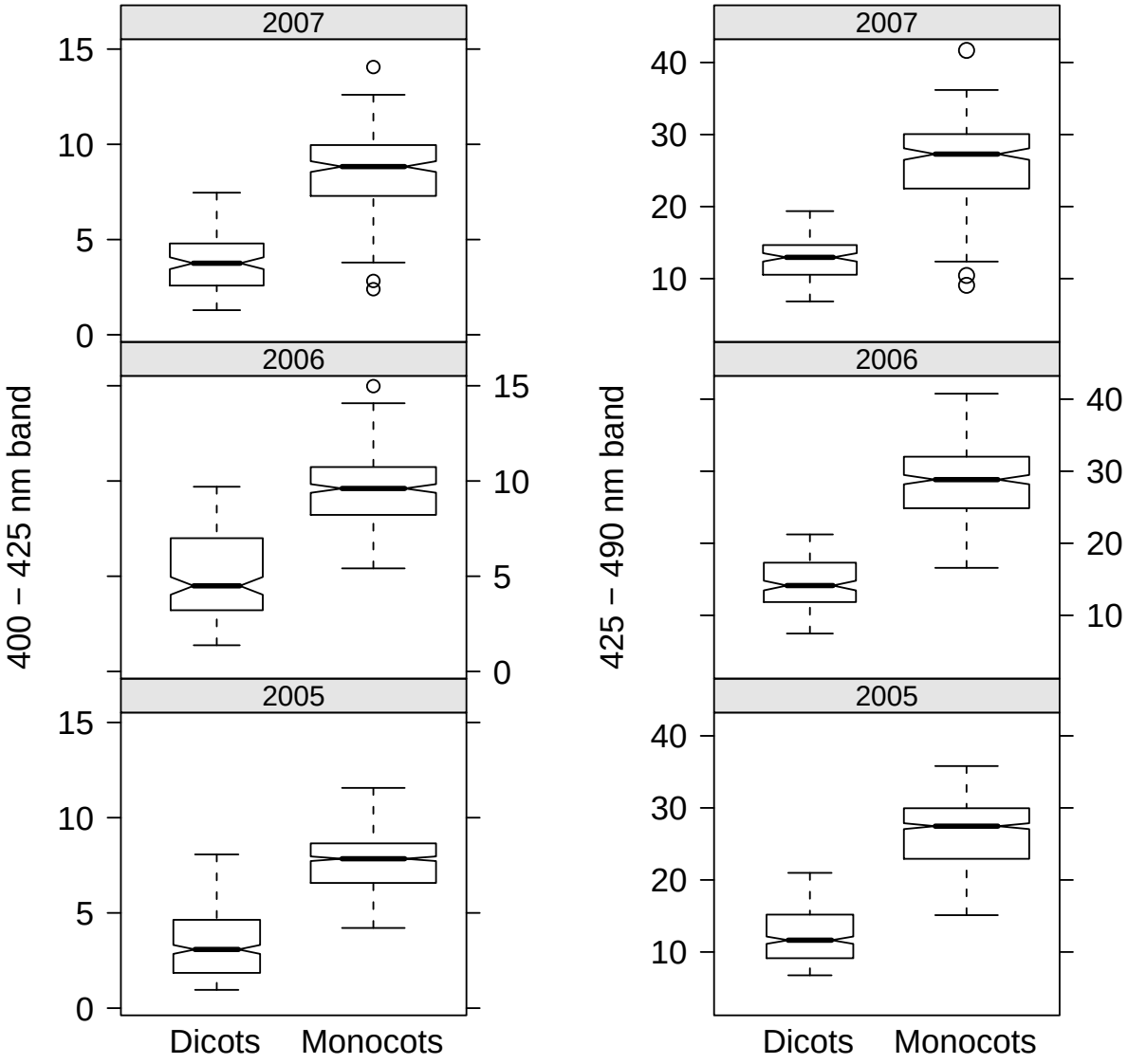

Figure 5: Boxplots of 2 band normalized data for each year.

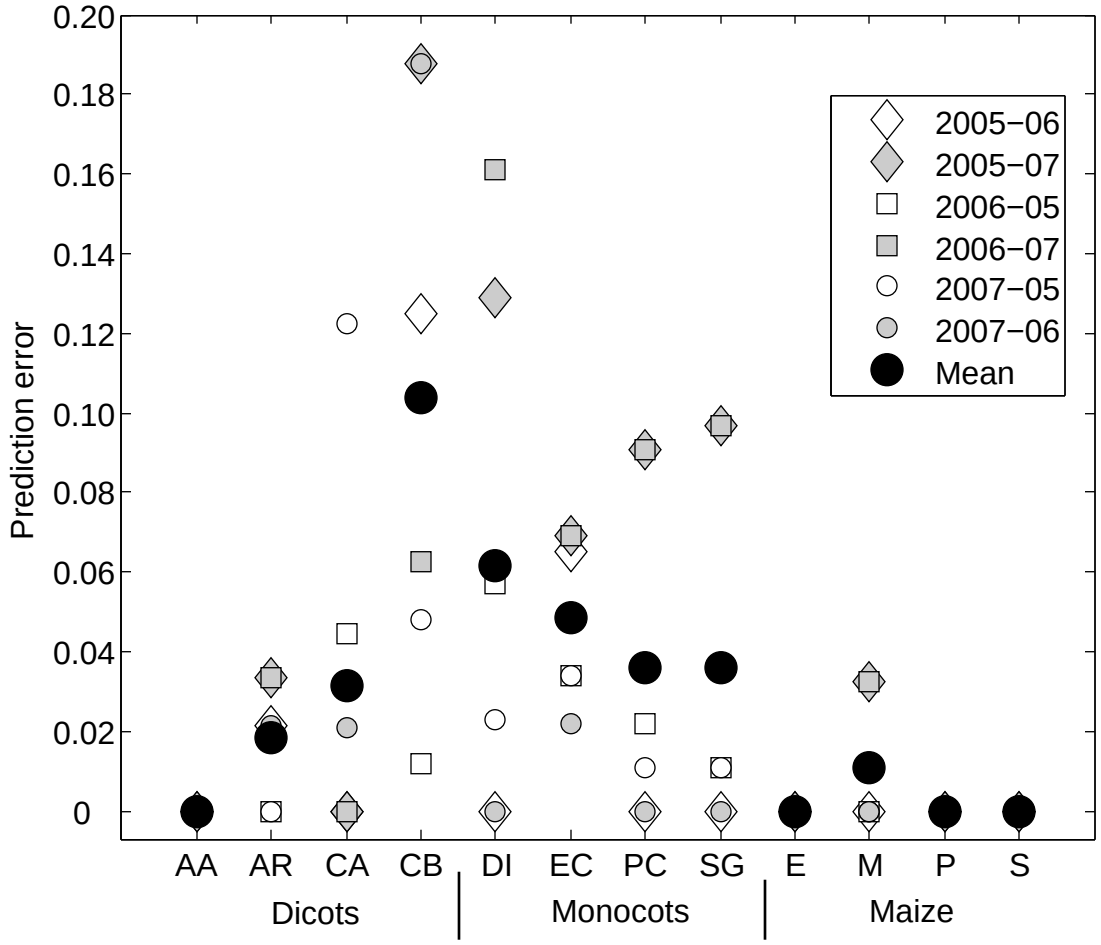

Figure 6: Error (fraction of misclassified) distribution across plants for the 6 inter annual validation runs with 2 normalized bands. In the legend 2005-06 means that the LDA model was calibrated using 2005 data and tested using 2006 data. The abbreviations labeling the horizontal axis refer to the code given in Table 1.

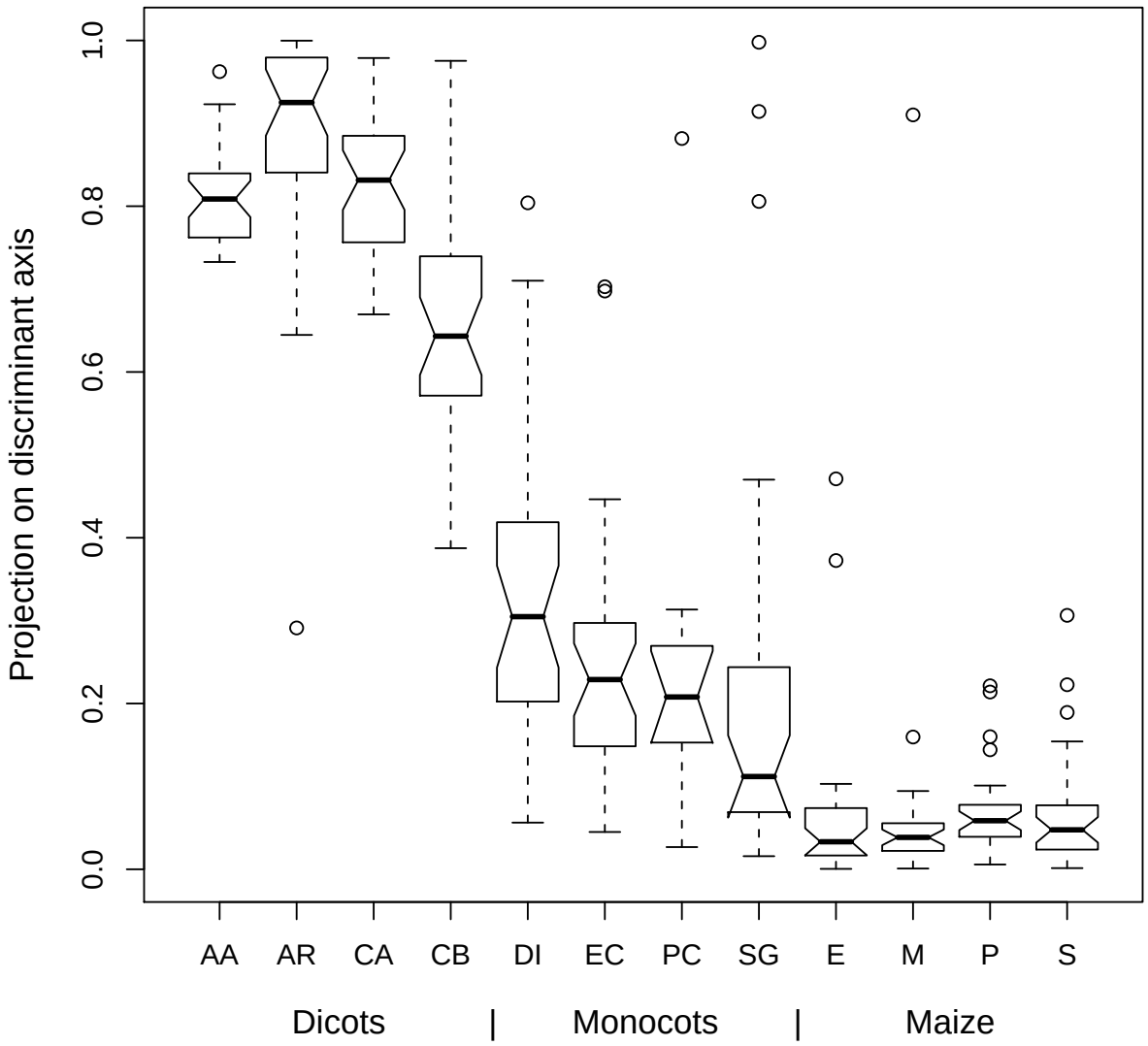

Figure 7: Boxplots of projection of the 2007 data on the discriminant axis defined using the 2005 data. The abbreviations labeling the horizontal axis refer to the code given in Table 1.