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1 PROSPECT-D: towards modeling leaf optical properties through a complete lifecycle

2 J.-B. Féret¹, A.A. Gitelson^{2,3}, S.D. Noble⁴, S. Jacquemoud⁵

3 ¹Irtsea, UMR TETIS, Maison de la Télédétection, 500 Rue Jean François Breton, 34000 Montpellier,
4 France

5 ²Faculty of Civil and Environmental Engineering, Israel Institute of Technology, Technion City, Haifa,
6 Israel

7 ³School of Natural Resources, University of Nebraska, Lincoln, USA

8 ⁴College of Engineering, University of Saskatchewan, 57 Campus Drive, Saskatoon, SK, S7N 5A9,
9 Canada

10 ⁵Institut de physique du globe de Paris - Sorbonne Paris Cité, Université Paris Diderot, UMR CNRS
11 7154, Case 7071, 35 rue Hélène Brion, 75013 Paris, France

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13 **Keywords:** Anthocyanins, Pigments, Hyperspectral, Leaf optical properties, PROSPECT, Radiative
14 transfer model

15 **Abstract**

16 Leaf pigments provide valuable information about plant physiology. High resolution monitoring of
17 their dynamics will give access to better understanding of processes occurring at different scales, and
18 will be particularly important for ecologists, farmers, and decision makers to assess the influence of
19 climate change on plant functions, and the adaptation of forest, crop, and other plant canopies. In
20 this article, we present a new version of the widely-used PROSPECT model, hereafter named
21 PROSPECT-D for dynamic, which adds anthocyanins to chlorophylls and carotenoids, the two plant
22 pigments in the current version. We describe the evolution and improvements of PROSPECT-D
23 compared to the previous versions, and perform a validation on various experimental datasets. Our
24 results show that PROSPECT-D outperforms all the previous versions. Model prediction uncertainty is
25 decreased and photosynthetic pigments are better retrieved. This is particularly the case for leaf
26 carotenoids, the estimation of which is particularly challenging. PROSPECT-D is also able to simulate
27 realistic leaf optical properties with minimal error in the visible domain, and similar performances to
28 other versions in the near infrared and shortwave infrared domains.

29 1. Introduction

30 Climate change is expected to affect vegetation worldwide, influencing air temperature,
31 biogeochemical cycles, and the frequency and intensity of plant disease. Leaf pigments are key
32 components of life on Earth: they are major contributors to individual plant health via complex
33 mechanisms allowing photosynthesis, plant growth, protection, adaptation to environmental
34 changes and phenological events. Their dynamics directly affects nutrient, nitrogen, carbon and
35 water cycles (Shiple et al., 2006; Joiner et al., 2011). Leaf pigments are therefore good indicators of
36 changes in environmental conditions from local to global scales.

37 Three main families of pigments are found in leaves: chlorophylls, carotenoids, and
38 anthocyanins. Chlorophyll-*a* and -*b*, the two types of molecules in higher plants, are the
39 fundamental light-absorbing pigments involved in photosynthesis. Carotenoids are accessory
40 pigments that contribute to light-harvesting and they also have essential photo-protective
41 properties. If xanthophylls and carotenes are the two major divisions of the carotenoid group, more
42 than 700 naturally occurring molecules have been identified so far, mainly in tissues other than
43 leaves (Britton et al., 2004). Anthocyanins are part of the flavonoid family: they are closely associated
44 with the colors in the autumn foliage of deciduous plant species. The term anthocyanin (*anthos* being
45 Greek for flower, and *kyanos* for blue) has been used since Marquart (1835) to represent the coloring
46 matter responsible for the various colors found in flowers, fruits and foliage in many plant species.
47 The exception are the core Caryophyllales, most of which produce betalains (betacyanins and
48 betaxanthins) (Brockington et al., 2011). While these betalain-producing families do include several
49 species of notable agronomic interest, both crops and weeds, they represent a small fraction of
50 plants investigated in a remote sensing context and are not considered in this work.

51 The anthocyanins include over 500 molecules, which accumulate in the vacuoles of various
52 cells and tissues. They create a pink, red, purple or blue coloration in the tissue depending on the
53 molecule, temperature, pH, and the presence or not of other molecules that may interact with them
54 (Davies, 2004; Gould et al., 2009). Their role is still not fully understood and described. For example,

55 until recently their biosynthesis during senescence was suspected to be nature's extravagancy
56 without a vital function, resulting from evolution in the absence of selection (Matile, 2000), and the
57 reason for their presence was explained as a by-product of the flavonoid biosynthetic pathway.
58 However, research over the past twenty years has demonstrated multiple functional implications of
59 these pigments during the plant life cycle, to the point that they have been called the "Nature's Swiss
60 army knife" by Gould (2004). Among the identified functions of anthocyanins are the protection of
61 the photosynthetic apparatus from damage due to excess light (Lee and Gould, 2002), environmental
62 stresses such as freezing or air pollution, plant pathogens, and predation (Lev-Yadun and Gould,
63 2008). An exhaustive review of the role of anthocyanins in plant leaves can be found in (Davies, 2004)
64 and (Gould, 2004).

65 Leaf pigments interact with solar radiation and change in response to environmental
66 conditions to optimize plant metabolism following complex pathways, within constraints of available
67 resources and stressors. As a result, leaf optical properties are directly impacted by the composition
68 of pigments. Remote sensing has proved to be a particularly suitable tool for the estimation of leaf
69 pigments, both at level of the leaf (e.g., Féret et al., 2008; Gitelson et al., 2006; le Maire et al., 2004;
70 Richardson et al., 2002; Sims and Gamon, 2002) and the canopy (e.g., Asner et al., 2015b; Atzberger
71 et al., 2010; Gitelson et al., 2005; Haboudane, 2004; Hmimina et al., 2015). The retrieval of pigment
72 content from remote sensing data involves two main approaches. The first approach is data-driven. It
73 includes univariate statistical models derived from spectral indices (e.g., Gitelson et al., 2006),
74 multivariate statistical models such as partial least squared regressions (e.g., Asner and Martin, 2009)
75 and machine learning algorithms (e.g., Verrelst et al., 2015). The relationships derived from these
76 predictive models are usually established empirically; they strongly depend on the variability and
77 quality of the data used to adjust these models, therefore they may lack robustness. The second
78 approach is based on radiative transfer models that exist both at leaf and canopy scales. Leaf models
79 generally simulate their spectral directional-hemispherical reflectance and transmittance while
80 canopy models simulate their spectral and bidirectional reflectance assuming that the leaf and soil

81 optical properties, the vegetation architecture, and the conditions of acquisition are known. The
82 combination of the two approaches (data-driven and physical) is also becoming increasingly popular
83 because it provides alternatives to extensive data collection required by the first, and high
84 computational resources required by the second (Féret et al., 2011; Verrelst et al., 2015).

85 To date, most studies devoted to vegetation pigments have focused on chlorophyll. This can be
86 explained by multiple factors. First, leaf chlorophyll content is the variable that has the strongest
87 effect on canopy reflectance in the VIS, and is a valuable proxy of nitrogen content and gross primary
88 production (e.g., Gitelson et al., 2012; Peng and Gitelson, 2012), which have strong impact in terms
89 of food and biomass production globally. Second, leaf chlorophyll content can be estimated with
90 relatively good accuracy using simple statistical regression models based on the relationship between
91 this variable and various spectral indices (e.g. Féret et al., 2011; Gitelson et al., 2006; le Maire et al.,
92 2004). Third, the availability of physical models including chlorophyll as input parameters allowed
93 investigating and better understanding its influence on the signal measured by satellite sensors,
94 leading to improved predictive models for leaf and canopy chlorophyll content in a more systematic
95 way than experimental data collection would have permitted. This is the case of the combined
96 PROSPECT leaf optical properties model (Jacquemoud and Baret, 1990) and SAIL canopy bidirectional
97 reflectance model (Verhoef, 1984; Verhoef et al., 2007), also referred to as PROSAIL, which has been
98 used for more than 25 years (Jacquemoud et al., 2009).

99 Current challenges such as food security, global warming and massive biodiversity loss now
100 require fine monitoring of vegetation status, with a level of information beyond inputs provided by
101 chlorophyll content alone. We need to address various issues related to vegetation, including stress,
102 invasive species, plant diseases and photosynthetic phenology, which implies monitoring the
103 dynamic of various pigment types such as carotenoids (Gamon et al., 2016) and anthocyanins. One of
104 the most studied “pigment-related” indicators derived from remote sensing is the Photochemical
105 Reflectance Index (PRI, Gamon et al., 1992) based on two narrow spectral bands in the green
106 spectrum: the PRI related to the xanthophyll cycle in the leaf; it captures the physiological response

107 of vegetation in response to a short term environmental stress inducing slight changes in
108 photosynthetic activity (Gamon et al., 1997, 1992, 1990). However, the primary driver of the PRI over
109 long time periods, at both leaf and canopy scales, is not the xanthophyll cycle, but rather the
110 changing leaf carotenoid pigment pool, typically expressed as the changing ratio of chlorophyll to
111 carotenoid pigments (or its inverse) (Filella et al., 2009; Nakaji et al., 2006; Styliniski et al., 2002).
112 Therefore, information about pigment content in vegetation is crucial when monitoring
113 photosynthetic phenology. At local scale, estimating pigment content from individuals using portable
114 devices, close range remote sensing or UAV can be useful for monitoring purposes in precision
115 agriculture or ecophysiology. At global scale, the ability to precisely monitor photosynthetic
116 phenology with satellite imagery is extremely valuable and would provide important input for models
117 of global terrestrial carbon uptake. Few studies focus on the direct estimation of carotenoids at leaf
118 scale (Chappelle et al., 1992; Gitelson et al., 2006, 2001; Sims and Gamon, 2002) and canopy scale
119 (Asner et al., 2015a, 2015b; Gamon et al., 2016; Hernández-Clemente et al., 2014, 2012; Ustin et al.,
120 2009; Zarco-Tejada et al., 2013). Even fewer focus on the estimation of anthocyanin, also at leaf
121 (Gamon and Surfus, 1999; Gitelson et al., 2001, 2006; Sims and Gamon, 2002; Steele et al., 2009) and
122 canopy (Rundquist et al., 2014) scales. The presence of overlapping features in the specific
123 absorption coefficient (SAC) of carotenoids and anthocyanins makes it difficult to separate and
124 quantify these accessory pigments using basic methods such as spectral indices, especially at canopy
125 scale (Ustin et al., 2009).

126 Monitoring vegetation status, stress, and shifts in the ecosystem functional properties is
127 critical. It will require sophisticated methods of leaf pigment content estimation, possibly combined
128 with the next generation of high resolution imaging spectrometers like Hypsiri (NASA), EnMAP (DLR)
129 or Hypxim (CNES), and vegetation radiative transfer models that incorporate all major pigments.
130 Present leaf optical properties models do not include pigments other than chlorophylls and
131 carotenoids, limiting the application of the physical approach to the study of these pigments
132 (Blackburn, 2007). SLOP (Maier et al., 1999), PROSPECT-5 (Féret et al., 2008), and soon after LIBERTY,

133 specifically designed for pine needles (Di Vittorio, 2009), are the three models that use carotenoids.
134 The dorsiventral leaf model designed by Stuckens et al. (2009) also differentiates chlorophylls from
135 carotenoids, but the SACs are those used in PROSPECT-5.

136 This article introduces a new version of the widely-used PROSPECT model, called PROSPECT-D,
137 which for the first time includes all three main pigments that control the optical properties of fresh
138 leaves, i.e., chlorophylls, carotenoids, and anthocyanins. The suffix -D stands for "dynamic" because
139 the model makes it possible to simulate leaf optical properties through a complete lifecycle, from
140 emergence, to anthocyanin-expressing stress responses, through to senescence. Given the success
141 and widespread use of PROSPECT-5, a major requirement for the development of PROSPECT-D was
142 to preserve, and ideally to improve on, the performance of PROSPECT-5 for pigment estimation in
143 samples containing little or no anthocyanin, while adding support for those that do. We performed a
144 new calibration of the SAC of each pigment, and updated the refractive index used by the model.
145 PROSPECT-D was then tested on several datasets displaying many plant species with a large range of
146 leaf traits including pigment composition. We evaluated its performance using two criteria resulting
147 from iterative optimization of leaf chemical and structural properties: the difference between the
148 measured and the modeled leaf directional-hemispherical reflectance and transmittance spectra,
149 and the accuracy of corresponding pigment estimation. We compared these two criteria for the new
150 and current versions of the model.

151

152 **2. Calibration of the model**

153 Several strategies for the calibration of PROSPECT have been investigated since its first version.
154 All of the procedures have points in common, such as the adjustment of one or several optical
155 constants (SAC of leaf chemical constituents, refractive index) over the VIS, near infrared and
156 shortwave infrared (SWIR) domains. A standard method consists in determining the optical constants
157 individually or simultaneously at each wavelength by using an iterative procedure (Féret et al., 2008;
158 Li and Wang, 2011). So far, there is no unique method, and adaptations have been proposed to

159 calibrate PROSPECT: Malenovský et al. (2006) adjusted SACs for needle-shaped leaves; Féret et al.
160 (2008) have simultaneously determined the refractive index and SAC of leaf constituents; Chen and
161 Weng (2012) have computed an individual refractive index for each leaf sample. In this section, we
162 provide information about the calibration of PROSPECT-D, including the selection of the calibration
163 dataset, as well as the main steps leading to updated optical constants.

164 2.1. Available datasets

165 Six independent datasets collected by several researchers for diverse purposes have been used
166 in this study (Table 1). They share directional-hemispherical reflectance and/or transmittance
167 spectra, and at least two pigments out of three (chlorophylls, carotenoids, and anthocyanins)
168 measured using wet chemistry. The ANGERS dataset includes more than 40 plant species; all the
169 other datasets are monospecies: European hazel (*Corylus avellana* L.) in HAZEL, Norway maple (*Acer*
170 *platanoides* L.) in MAPLE, Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch) in VIRGINIA, and
171 Siberian dogwood (*Cornus alba* L.) in DOGWOOD-1 and -2. Table 1 summarizes the spectral and
172 chemical information available for each dataset. Additional information about the protocols used to
173 conduct the experiments, collect the leaves, measure their optical properties and determine their
174 wet chemistry can be found in (Féret et al., 2008; Gitelson et al., 2009, 2006, 2001; Merzlyak et al.,
175 2008). Note that DOGWOOD-2 does not contain transmittance spectra; that the total chlorophyll
176 content (C_{ab} , expressed in $\mu\text{g cm}^{-2}$) is available in all datasets; that the total carotenoid content (C_{xc} ,
177 expressed in $\mu\text{g cm}^{-2}$) and the total anthocyanin content (C_{anth} , expressed in $\mu\text{g cm}^{-2}$) have not been
178 determined in HAZEL and ANGERS, respectively. Finally, the 400-780 nm spectral range used for the
179 calibration of the pigment content is common to all datasets except DOGWOOD-1.

180

181 **Table 1.** Description of the leaf datasets used in this study. * The ANGER dataset is available online <http://opticleaf.ipgp.fr/index.php?page=database>.

| Database | Reference | Spectral range (nm) | Number of leaves | Optical properties | Chlorophyll content C_{ab} ($\mu\text{g cm}^{-2}$) | | | Carotenoid content C_{xc} ($\mu\text{g cm}^{-2}$) | | | Anthocyanin content C_{anth} ($\mu\text{g cm}^{-2}$) | | |
|-----------|-----------|---------------------|------------------|--------------------|---|-------|--------|--|------|-------|---|------|-------|
| | | | | | Mean \pm SD | Min | Max | Mean \pm SD | Min | Max | Mean \pm SD | Min | Max |
| ANGERS* | 1, 2 | 400-2500 | 308 | R & T | 34.41 \pm 21.85 | 0.78 | 106.70 | 8.84 \pm 5.14 | 0.00 | 25.28 | N/A | N/A | N/A |
| VIRGINIA | 3, 4 | 400-800 | 81 | R & T | 11.05 \pm 14.60 | 0.09 | 53.76 | 2.98 \pm 3.06 | 0.15 | 12.27 | 8.63 \pm 10.77 | 0.00 | 37.50 |
| MAPLE | 3, 4, 6 | 400-780 | 48 | R & T | 7.43 \pm 7.36 | 0.14 | 32.98 | 5.25 \pm 2.37 | 1.82 | 10.40 | 8.75 \pm 6.83 | 1.12 | 21.66 |
| DOGWOOD-1 | 3, 4, 5 | 440-796 | 20 | R & T | 4.53 \pm 4.84 | 0.07 | 15.03 | 2.96 \pm 2.06 | 0.42 | 5.71 | 6.88 \pm 5.52 | 0.40 | 15.49 |
| HAZEL | 3, 4 | 400-800 | 13 | R & T | 26.37 \pm 3.55 | 22.69 | 34.62 | N/A | N/A | N/A | 7.13 \pm 4.19 | 0.25 | 13.61 |
| DOGWOOD-2 | 6 | 400-1000 | 51 | R | 23.77 \pm 7.58 | 1.53 | 39.81 | 5.39 \pm 2.26 | 1.73 | 10.76 | 12.71 \pm 8.21 | 1.07 | 30.23 |

182 1: Féret et al. (2008); 2: Féret et al. (2011); 3: Merzlyak et al. (2008); 4: Gitelson et al. (2009); 5: Gitelson et al., (2001); 6: Gitelson et al. (2006).

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2.2. Data selection for calibration

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The calibration of PROSPECT-D requires the leaf samples to have general properties at a minimal level: *i*) pigment content expressed in the same unit for chlorophylls, carotenoids, and anthocyanins, and *ii*) reflectance and transmittance spectra in the 400-780 nm wavelength range. A challenge to calibrating a high performance leaf optical properties model is the lack of comprehensive datasets meeting these two criteria. Among those available, only VIRGINIA and MAPLE fulfill these conditions. Preliminary calibration tests using part or all of these datasets led to SACs with strong discrepancies and poor performances for the estimation of pigment content. Therefore we considered alternative methods combining different data sources and expanding the pool of available calibration data to fill in the gaps. Féret et al. (2008) exploited the ANGERS dataset to calibrate PROSPECT-4 and -5. It is characterized by a wide range of leaf types and pigment contents, and it proved to be well suited for the determination of the SAC of the chlorophyll and carotenoid pigments. These desirable properties of ANGERS come from the variety of leaf types: while chlorophyll and carotenoids contents are usually highly correlated in mature leaves, ANGERS includes juvenile, stressed and senescent leaves lowering this correlation and allowing the SAC of each of these pigments to be adjusted independently from the others, despite their overlapping domain of absorption. Therefore we took the decision to include ANGERS in the calibration dataset and to estimate the corresponding C_{anth} using a spectral index. To avoid the associated uncertainty leading to errors in the SACs, we combined a subset of ANGERS with a subset of VIRGINIA that included accurate measurements of C_{anth} obtained by wet chemistry (Merzlyak et al., 2008). Leaves from VIRGINIA were collected in a park at Moscow State University; they contained very high levels of anthocyanin and low to moderate levels of chlorophyll and carotenoids; they displayed the maximum range of anthocyanin among all the available datasets. In this section, we first explain how C_{anth} was estimated in ANGERS, and then how we split ANGERS and VIRGINIA into calibration and

209 validation subsets. Finally, we present a sensitivity study intended to analyze the influence of the
 210 expected C_{anth} uncertainty in ANGERS on the performances of the model.

211

212 **2.2.a. Estimation of leaf anthocyanin content in ANGERS**

213 Several nondestructive methods to estimate C_{anth} from leaf reflectance have been identified
 214 and tested on experimental data for which the anthocyanin content has been measured. These
 215 methods included spectral indices and machine learning algorithms such as support vector regression
 216 (Gitelson et al., 2001, 2006, 2009; van den Berg and Perkins, 2005; Pfündel et al., 2007). The modified
 217 Anthocyanin Reflectance Index (*mARI*) designed by Gitelson et al. (2006) led to the best results
 218 when using a leave-one-out cross-validation. This index is defined by:

219

220

$$mARI = (R_{green}^{-1} - R_{red\ edge}^{-1}) \times R_{NIR} \quad \text{Eq. 1}$$

221

222 where R_{green} is the mean reflectance between 540 nm and 560 nm, $R_{red\ edge}$ the mean reflectance
 223 between 690 nm and 710 nm, and R_{NIR} the mean reflectance between 760 nm and 800 nm. We first
 224 studied the relationship between *mARI* and C_{anth} over the 213 samples for which information about
 225 anthocyanins was available, i.e., all datasets of Table 1 with the exception of ANGERS. We found a
 226 strong linear relationship for *mARI* values smaller than 5 (137 samples, $R^2 = 0.90$, $RMSE = 1.18 \mu g$
 227 cm^{-2}) and a weak one for *mARI* values greater than 5 (76 samples, $R^2 = 0.37$, $RMSE = 6.35 \mu g$
 228 cm^{-2}) (Figure 1). These samples with $mARI < 5$ correspond to mature green, yellow, and reddish/red
 229 leaves with C_{anth} values less than $12 \mu g$ cm^{-2} . Leaves with C_{anth} values higher than $12 \mu g$ cm^{-2} are
 230 generally dark red and contain small amounts of chlorophyll. In that case, absorptance between 540
 231 nm and 560 nm exceeded 90% and further increases of anthocyanin did not change leaf optical
 232 properties. A linear model for anthocyanin estimation (Eq. 2) was then derived from the subset
 233 excluding the samples with $mARI > 5$ and $C_{anth} > 12 \mu g$ cm^{-2} .

234

$$C_{anth} = 2.11 \times mARI + 0.45 \quad \text{Eq. 2}$$

235

236 Eq. 2 was applied to the ANGERS dataset to determine C_{anth} . The anthocyanin content ranged237 from 0 to $17.1 \mu\text{g cm}^{-2}$, with a mean value of $1.7 \mu\text{g cm}^{-2}$.

238

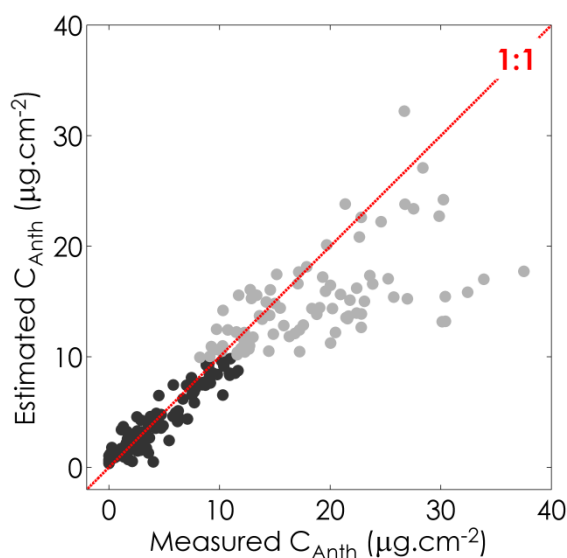


Figure 1. Relationship between C_{anth} obtained from wet chemistry and C_{anth} estimated from reflectance data after application of Eq. 2. The black dots correspond to the 137 leaf samples with $mARI < 5$ ($R^2 = 0.90$) and the grey dots correspond to the 76 leaf samples with $mARI > 5$ ($R^2 = 0.37$). Eq. 2 was adjusted only on the black dots.

239

2.2.b. Selection of the calibration samples

240 In order to keep as many samples as possible, we decided to build a calibration dataset made of leaf

241 samples selected both in ANGERS and VIRGINIA. The influence of the uncertainty associated to C_{anth} 242 in ANGERS is expected to be mitigated by the accuracy of C_{anth} in VIRGINIA.243 In VIRGINIA we identified samples characterized by low C_{ab} ($< 20 \mu\text{g cm}^{-2}$) and C_{xc} ($< 5 \mu\text{g cm}^{-2}$)

244 in order to decrease the combined influence of the pigments on leaf optical properties, but also to

245 minimize the correlation among pigments. It should allow capturing the influence of anthocyanins

246 independently from the other pigments. We randomly selected 20 samples, leaving a total of 61
247 samples of VIRGINIA for the validation.

248 In ANGERS we discarded at first 14 atypical samples, the spectral behavior of which was
249 incompatible with PROSPECT assumptions. For example, we removed samples collected on
250 *Eucalyptus gunnii* and *Cornus alba*, the overall reflectance of which was very high in the VIS because
251 of the presence of wax (Barry and Newnham, 2012); and three samples of *Schefflera arboricola*
252 displaying uncharacteristic optical properties in the blue (400-450 nm). We also removed samples
253 with $mARI > 5$ as the uncertainty associated to C_{anth} was particularly high (Figure 1). Finally we
254 eliminated leaf samples that had little influence on the calibrated SACs. For that purpose we
255 determined a reference SAC for each pigment using the 314 preselected leaf samples (20 from
256 VIRGINIA and 294 from ANGERS). The ANGERS samples inducing changes in the SACs higher than 5%
257 between 425 nm and 475 nm were kept in the calibration datasets, the others were transferred to
258 the validation dataset.

259 In total, a dataset named CALIBRATION and combining subsets of ANGERS (144 samples) and
260 VIRGINIA (20 samples) was used for the calibration phase.

261 **2.2.c. Sensitivity of the calibration to the uncertainty associated with C_{anth} in ANGERS**

262 As abovementioned, determining C_{anth} with a spectral index like $mARI$ leads to uncertainty likely to
263 impact the quality of the calibration. We performed a sensitivity analysis with the aim of
264 understanding the influence of this uncertainty on the SACs and on the overall performances of the
265 model. It consisted in adding a Gaussian noise ($\sigma = 1.18 \mu\text{g cm}^{-2}$) to C_{anth} in ANGERS prior to the
266 calibration and validation procedure. We repeated the operation 50 times.

267

268 **2.3. Selection of the refractive index**

269 PROSPECT is based on the generalized plate model proposed by (Allen et al., 1970, 1969). A
270 plant leaf is modeled as a pile of elementary layers characterized by an absorption coefficient and a
271 refractive index provided at a given wavelength. In the first version of the model, Jacquemoud and

272 **Baret (1990)** used an albino maize leaf to obtain an experimental spectrum of the refractive index for
273 the elementary layers. **le Maire et al. (2004)** and **Féret et al. (2008)** adopted a new strategy based on
274 numerical optimization to determine the optical constants of PROSPECT at the same time. Their
275 refractive index performed slightly better than the previous versions at estimating leaf chemical
276 constituents, but the strong spectral variations observed in the visible wavelengths induced small
277 artifacts in the optical properties of leaves displaying high pigment content. **Stuckens et al. (2009)**
278 also adjusted a unique refractive index for all leaves.

279 Attempts to obtain a unique refractive index spectrum for all leaves are actually unfounded
280 and inconsistent with the Kramers-Kronig relations that state that the real (refractive index) and
281 imaginary (absorption coefficient) parts of the complex refractive index of a medium are physically
282 linked (**Lucarini et al., 2005**). These relations allow direct computation of the refractive index of a
283 medium based on its absorption properties on an extended spectral domain. **Chen and Weng (2012)**
284 used the Kramers-Kronig relations to derive an effective refractive index adjusted to each leaf
285 sample, obtaining very promising results. However, leaf chemical and spectral databases are often
286 incomplete; in particular they cover a limited range of the electromagnetic spectrum, so such a
287 method is impracticable. As a consequence we considered the refractive index to be independent of
288 the leaf sample in this study, but changed strategy compared to PROSPECT-5 in order to avoid the
289 abovementioned artifacts resulting from numerical optimization. Two options were tested: 1) using
290 the refractive index imbedded in PROSPECT-3, and 2) taking the average refractive index derived
291 from minimum and maximum values computed by **Chen and Weng (2012)** and corresponding to the
292 boundaries of the grey area in **Figure 2**. The spectra displayed in **Figure 2** strongly differ in shape in
293 the VIS: the overall profile of the refractive indices computed by **Chen and Weng (2012)** is quite
294 similar to that measured for pure liquid water (**Hale and Querry, 1973**), gradually decreasing from the
295 visible to the infrared, whereas the indices in PROSPECT-3 and -5 are very much alike in the near and
296 shortwave infrared (1000-2500 nm) and show a steeper decrease. Divergence between the refractive
297 index derived from **Chen and Weng (2012)** and those used in PROSPECT-3 and -5 strongly increases

298 with wavelength. We performed the full calibration of PROSPECT (including optimal adjustment of
 299 SAC as described in Section 2.2.b) with each refractive index.
 300

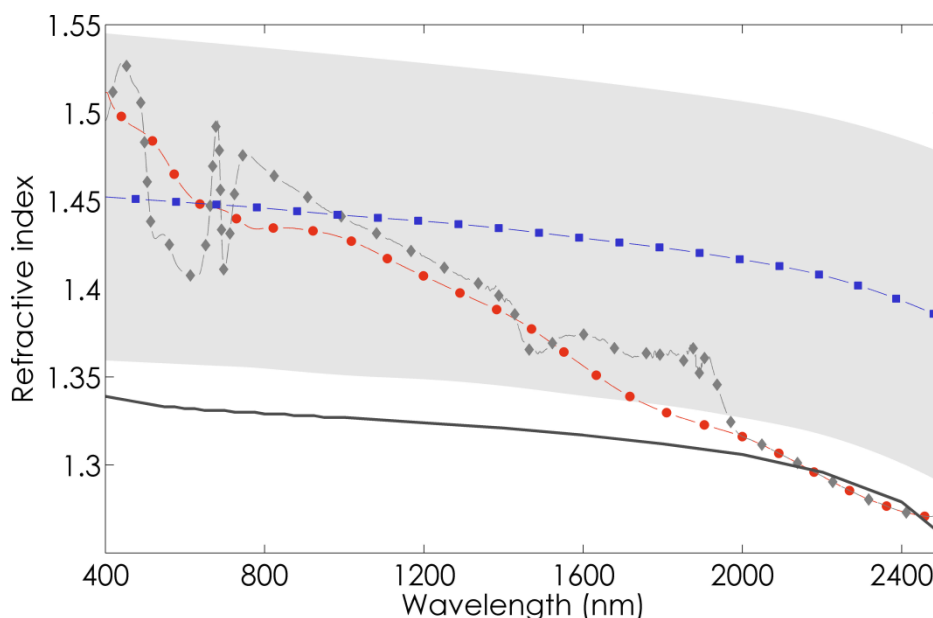


Figure 2. Comparison of the refractive index used in PROSPECT-3 (red dots), PROSPECT-5 (grey diamonds) and corresponding to the mean refractive index proposed by (Chen and Weng, 2012) (blue squares). The grey area corresponds to the range of variation of the refractive index proposed by Chen and Weng, (2012); the plain grey line corresponds to the refractive index for pure liquid water (Hale and Querry, 1973).

301

302 2.4. Optimal adjustment of the specific absorption coefficients

303 The adjustment of the SAC for each group of pigments is based on numerical optimization
 304 routines applied to experimental data. As in PROSPECT-5 we assumed that the chlorophyll *a:b* ratio
 305 was constant and we combined carotenes and xanthophylls in the carotenoid group. Similarly
 306 anthocyanins were assumed to include all types of anthocyanins contributing to light absorption in
 307 the VIS. Given the well-known sensitivity of the anthocyanin absorption properties to pH, which is
 308 due to a reversible structural change that occurs in the C ring of the molecule, this hypothesis may be

309 incorrect in certain situations. However, environment inside a vacuole is generally slightly acid, with
 310 pH values reported to fall within the range of 5.0 to 6.0 pH units, with a mean pH of 5.5 (Mathieu et
 311 al., 1989; Martinière et al., 2013).

312 Solved SAC values were constrained to be positive. To prevent erroneous absorption
 313 assignments, the wavelength domains were narrowed to 400-750 nm for chlorophylls, 400-560 nm
 314 for carotenoids, and 400-660 nm for anthocyanins. These ranges are broader than *in vitro* due to the
 315 detour effect: the lengthening of the optical path-length within the leaf results in substantial
 316 flattening of the absorption spectrum *in vivo* (e.g., Rühle and Wild, 1979; Fukshansky et al., 1993).

317 The calibration followed a two-steps algorithm described in Féret et al. (2008). First, we
 318 determined the structure parameter N_j of each leaf j in the calibration datasets on the basis of an
 319 iterative optimization: N_j was estimated based on a multivariate iterative optimization,
 320 simultaneously with three absorption coefficients using reflectance and transmittance values
 321 measured at three wavelengths corresponding to the minimum absorptance (λ_1), maximum
 322 reflectance (λ_2), and maximum transmittance (λ_3) of the leaf. These values are generally located on
 323 the NIR plateau. The iterative optimization was performed using the merit function:

$$M_{leafN}(N_j, k(\lambda_1), k(\lambda_2), k(\lambda_3)) = \sum_{l=1}^3 \left(R_{meas,j}(\lambda_l) - R_{mod}(N_j, k(\lambda_l)) \right)^2 + \left(T_{meas,j}(\lambda_l) - T_{mod}(N_j, k(\lambda_l)) \right)^2 \quad \text{Eq. 3}$$

326 with $R_{meas,j}(\lambda_l)$ and $T_{meas,j}(\lambda_l)$ the measured reflectance and transmittance of leaf j at the
 327 wavelength λ_l , R_{mod} and T_{mod} the modeled values, and $k(\lambda)$ the absorption coefficient of a
 328 compact layer at the wavelength λ , which is adjusted simultaneously with N_j . In Eq. 3, $k(\lambda)$ is not
 329 decomposed into specific absorption of the different chemical constituents. This step occurs in the
 330 NIR where pigments have little if any influence, so $k(\lambda)$ is primarily affected by water absorption.

331 The SAC to be calibrated were then computed by inverting PROSPECT on the $n = 164$ leaves of the
 332 calibration dataset. We minimized the merit function J at each wavelength:

333

$$\begin{aligned}
 & J\left(\{K_{spe,i}(\lambda)\}_{i=1:n}\right) \\
 = & \sum_{j=1}^n \left(R_{meas,j}(\lambda) - R_{mod,j}(N_j, k(\lambda)) \right)^2 \\
 & + \left(T_{meas,j}(\lambda) - T_{mod,j}(N_j, k(\lambda)) \right)^2
 \end{aligned}
 \tag{Eq. 4}$$

334

335 where

336

$$k(\lambda) = \frac{\sum_i K_{spe,i}(\lambda) \times C_{i,j}}{N_j}
 \tag{Eq. 5}$$

337

338 $k(\lambda)$ is the absorption coefficient of a compact layer at the wavelength λ , $K_{spe,i}(\lambda)$ is the SAC
 339 of constituent i , $C_{i,j}$ is the corresponding content for leaf j , N_j is the leaf structure parameter of leaf
 340 j , and n corresponds to the number of biochemical constituents for which the SACs are
 341 simultaneously calibrated.

342

343 3. Validation: datasets and criteria for the comparison of model performances

344 We performed model inversions on the validation dataset with PROSPECT-D, as well as
 345 PROSPECT-3 and -5. The performances of the different versions of the model were compared in
 346 terms of pigment content estimation and leaf spectra fit. The validation was performed using all leaf
 347 samples after exclusion of the calibration samples.

348 PROSPECT was inverted on the validation dataset using an iterative method for optimization. It
 349 consists in finding the best combination of leaf chemical and structural parameters that minimizes
 350 the merit function:

351

352

$$\begin{aligned}
 M_{inv}(N, \{C_i\}_{i=1:p}) &= \sum_{\lambda=1}^{n_\lambda} \left(R_{meas,\lambda} - R_{mod,\lambda}(N, \{C_i\}_{i=1:p}) \right)^2 \\
 &+ \left(T_{meas,\lambda} - T_{mod,\lambda}(N, \{C_i\}_{i=1:p}) \right)^2
 \end{aligned}
 \tag{Eq. 6}$$

353

354 with n_λ the number of available spectral bands, N the leaf structure parameter, C_i the content of
 355 constituent i , and p the number of leaf biochemical constituents. In this study, we simultaneously
 356 estimated the six input parameters of PROSPECT: pigments (C_{ab} , C_{xc} , C_{anth}), equivalent water
 357 thickness (EWT), leaf mass per area (LMA) and leaf structure parameter (N).

358 In essence, candidate sets of these parameters are iteratively input in forward mode and the
 359 resulting modeled reflectance and/or transmittance spectra compared against the measured ones;
 360 the parameter are revised until the minimum value of the merit function is found. When the
 361 assumptions of the model are not met, for example if a pigment is not taken into account, or if the
 362 SACs are incorrect, the inversion will converge towards a suboptimal solution, inducing errors in the
 363 estimation of the parameters. This situation occurs when we try to invert PROSPECT-3 on yellowing
 364 leaves; C_{ab} is overestimated to compensate for the absorption of C_{xc} .

365 The root mean square error ($RMSE$ expressed in $\mu\text{g cm}^{-2}$) can be computed to appraise the
 366 difference between the measured and estimated pigment content:

367

$$RMSE = \sqrt{\frac{\sum_{j=1}^n (X_{meas,j} - X_{mod,j})^2}{n}}
 \tag{Eq. 7}$$

368

369 where $X_{meas,j}$ are the measured values and $X_{mod,j}$ are the values estimated by model inversion for
 370 leaf j . As for the quality of the fit, it is appraised by the spectral RMSE which calculates the difference
 371 between the measured and simulated reflectance and transmittance spectra on a wavelength-by-
 372 wavelength basis.

373

374 **4. Results**

375 **4.1. Selection of a calibration dataset**

376 **Figure 3** shows the pigment distribution corresponding to the calibration and validation
 377 samples in ANGERS and VIRGINIA: note that the calibration samples display low to moderate C_{ab} and
 378 C_{xc} values, whereas the validation samples encompasses significantly broader pigment contents. The
 379 calibration samples with high C_{anth} come from VIRGINIA, for reasons explained earlier.

380

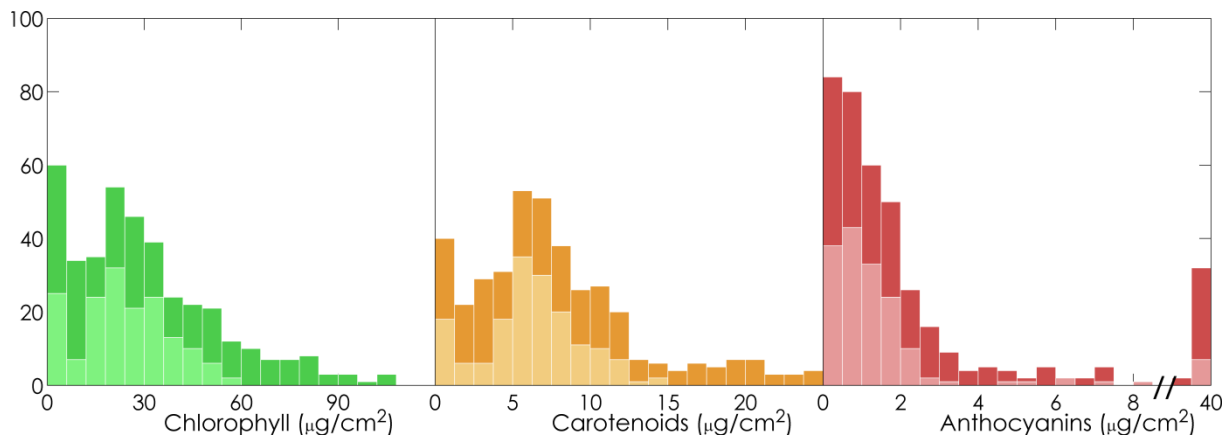


Figure 3. Stacked distribution of pigment content for the calibration and validation samples selected from ANGERS and VIRGINIA. Light colors: calibration (144 samples from ANGERS, 20 Samples from VIRGINIA); dark colors: validation (164 samples from ANGERS, 61 samples from VIRGINIA).

381

382 **4.2. Selection of the refractive index**

383 The refractive index spectra displayed in **Figure 2** provide advantages and disadvantages in
 384 terms of model accuracy both for the estimation of leaf chemistry and the simulation of leaf optical
 385 properties. We performed calibrations of PROSPECT-D as detailed in **Section 3**, using either the
 386 refractive index of PROSPECT-3 and the one derived by **Chen and Weng (2012)**. Then we inverted the
 387 two versions of the model on ANGERS, the only dataset covering the SWIR as well as including
 388 measurements of *EWT* and *LMA*. Overall the performances obtained for pigment retrieval and
 389 simulation of leaf optics were similar in the VIS. For water and dry matter, results were also very

390 similar. Simulated leaf reflectance and transmittance spectra also exhibited very slight differences in
 391 the VIS, while they were noticeable in the NIR and SWIR. This is illustrated by Figure 4 that displays
 392 the spectral RMSE calculated between measured and simulated leaf spectra for ANGERS. The
 393 reflectances are comparable, with slightly higher RMSE obtained with the refractive index derived
 394 from Chen and Weng (2012) between 1500 nm and 1900 nm; there are higher discrepancies in the
 395 transmittances. Based on these results, we assigned the refractive index used in PROSPECT-3 to
 396 PROSPECT-D.

397

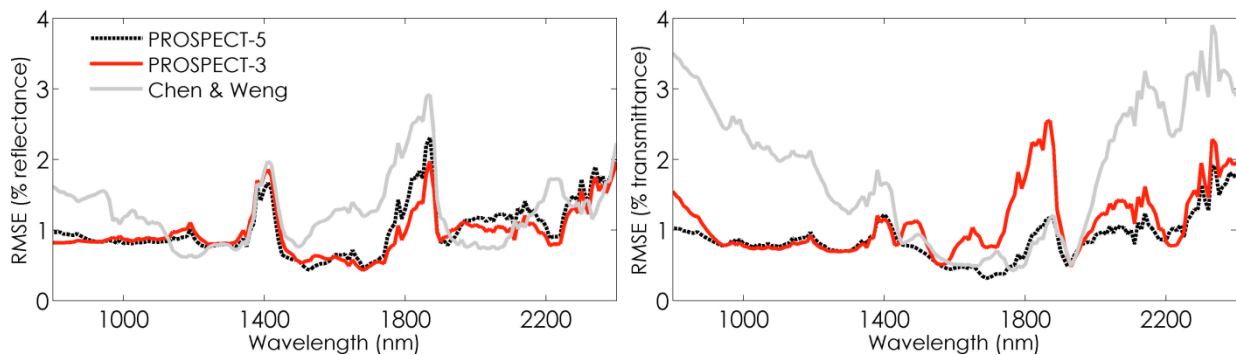


Figure 4. Spectral RMSE between measured leaf optics and simulated reflectance (left), and transmittance (right) obtained after model inversion with the refractive index used in PROSPECT-5 (black dotted line), PROSPECT-3 (red), and derived from Chen and Weng (2012) (light grey).

398

399 4.3. Adjustment of the specific absorption coefficients

400 Figure 5 displays the SACs of pigment in PROSPECT-5 and PROSPECT-D. The differences
 401 between the two models are minor for chlorophylls and carotenoids beyond 450 nm. However,
 402 noticeable differences can be observed for carotenoids between 400 nm and 450 nm: compared to
 403 PROSEPECT-D, the SAC of C_{xc} sharply increases towards the ultraviolet (UV) domain in PROSPECT-5,
 404 possibly due to the presence of flavonoids in the calibration samples. This augmentation is
 405 compensated by a slight decrease of the SACs of C_{ab} and C_{anth} . The SAC of anthocyanins shows a
 406 strong absorption between 450 nm and 650 nm, that peaks at about 550 nm. This result is in

407 agreement with the absorption spectra reported in the literature (Dougall and Baker, 2008; Peters
 408 and Noble, 2014). The broadness of the absorption peak may be caused by calibration artifacts
 409 related to residual correlations between the pigments.

410

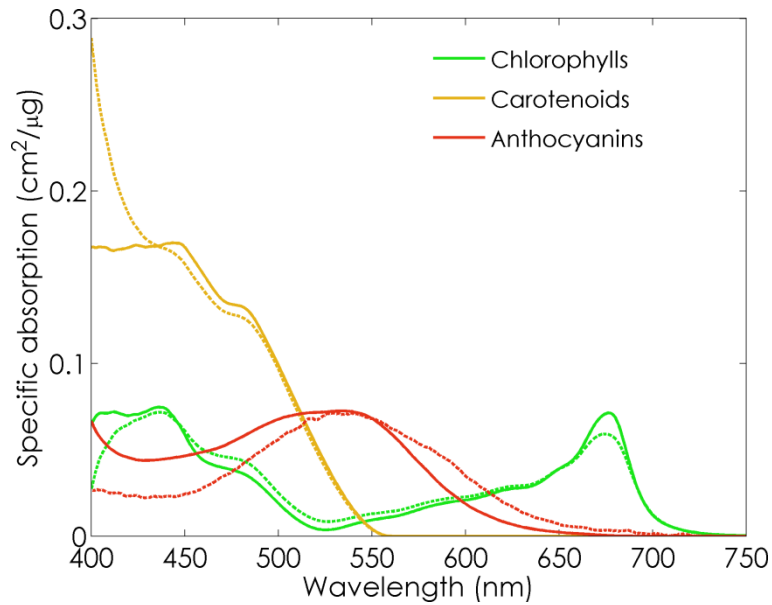


Figure 5. Specific absorption coefficients of chlorophylls (green), carotenoids (orange), and anthocyanins (red). The solids line correspond to the SAC of all the pigments in PROSPECT-D, the dashed lines to the SAC of chlorophylls and carotenoids in PROSPECT-5, and to the SAC of anthocyanins measured by Peters and Noble (2014) using thin layer chromatography.

411

412 4.4. Validation of model performances

413 4.4.a. Estimation of leaf pigment by PROSPECT inversion

414 C_{ab} , C_{xc} and C_{anth} have been estimated by inversion of PROSPECT-D on a dataset hereafter
 415 called VALIDATION. It gathered all experimental datasets (Table 1) except DOGWOOD-2 which did
 416 not contain transmittance spectra. ANGERS was also excluded for anthocyanins since C_{anth} has been
 417 indirectly estimated using a spectral index.

418 Table 2 compares the performances of PROSPECT-3, -5 and -D for pigment estimation in terms
 419 of RMSE. One can note a markedly improvement of C_{xc} estimation by PROSPECT-D for all datasets. In

420 ANGERS, which mainly contain green leaves, PROSPECT-5 and PROSPECT-D perform similarly at
 421 estimating C_{ab} , with a slight increase of RMSE obtained with PROSPECT-D. It can be explained by the
 422 fact that these samples correspond to calibration samples in PROSPECT-5. PROSPECT-D surpasses the
 423 previous versions of the model for all datasets other than ANGERS. Importantly, the new model is
 424 able to accurately estimate C_{xc} even in anthocyanic leaves, which has been problematic for other
 425 techniques.

426

427 **Table 2.** RMSE ($\mu\text{g cm}^{-2}$) of the estimation of leaf pigment content using PROSPECT-3 (P-3),
 428 PROSPECT-5 (P-5), and PROSPECT-D (P-D) inversion. The bold font for numbers indicates the lowest
 429 values.

| Database | C_{ab} | | | C_{xc} | | C_{anth} |
|----------------|----------|-------------|-------------|----------|--------------|------------|
| | P-3 | P-5 | P-D | P-5 | P-D | P-D |
| CALIBRATION | 6.84 | 3.69 | 3.43 | 6.69 | 1.31 | 1.74 |
| ANGERS (VAL) | 11.07 | 6.71 | 7.07 | 6.90 | 3.81 | 3.22 |
| MAPLE | 15.54 | 7.85 | 3.28 | 17.28 | 2.23 | 2.34 |
| VIRGINIA (VAL) | 14.38 | 7.74 | 2.95 | 17.41 | 1.19 | 4.54 |
| DOGWOOD-1 | 14.94 | 7.90 | 3.12 | 18.07 | 1.21 | 2.24 |
| HAZEL | 20.88 | 9.67 | 2.36 | n.a. | n.a. | 2.82 |
| DOGWOOD-2 | 37.22 | 16.51 | 6.08 | 20.92 | 10.92 | 14.39 |
| VALIDATION | 13.33 | 7.33 | 5.58 | 12.67 | 3.06 | 3.49 |

430

431 **Figure 6** illustrates the differences observed in **Tables 2**. PROSPECT-3 overestimated C_{ab} for all
 432 datasets, even with ANGERS which mainly contained green leaves; this overestimation was reduced
 433 when using PROSPECT-5 or PROSPECT-D. The substantial improvement in C_{xc} estimation when using
 434 PROSPECT-D is a significant result of this article: overall, the RMSE was divided by four compared to
 435 the results obtained with PROSPECT-5. The inversion of PROSPECT-5 on anthocyanin-rich leaves
 436 sometimes converged towards the upper bound on C_{xc} ($30 \mu\text{g cm}^{-2}$). It is likely that this
 437 overestimation of carotenoid content results from the strong absorption of light by anthocyanins in
 438 the same wavelength range as carotenoids, and this absorption is not properly modeled by
 439 PROSPECT-5. In ANGERS, the systematic underestimation of C_{xc} by PROSPECT-5 in leaves containing
 440 high amounts of photosynthetic pigments was greatly reduced by PROSPECT-D. The overestimation

441 of C_{xc} in anthocyanin-rich leaves was also corrected. The lower performances of PROSPECT-D on
 442 DOGWOOD-2 are probably explained by the absence of transmittance spectra. As explained in
 443 **Section 4.1**, the samples selected for the calibration dataset in ANGERS were marked out by low to
 444 medium pigment content, therefore they were not representative of the full range of variation found
 445 in this dataset: nevertheless, this did not prevent us from estimating high pigment content with
 446 accuracy. This is explained by slight changes in the optical properties of leaves with high-pigment
 447 content when carotenoids increase, due to saturation effects. It also suggests that the physical
 448 description used in PROSPECT is correct, as its ability to estimate pigment content goes beyond the
 449 range used for calibration. Samples showing underestimated C_{xc} in ANGERS were discarded from the
 450 calibration dataset due to unusual optical properties (surface effects) or very high $mARI$.

451

452

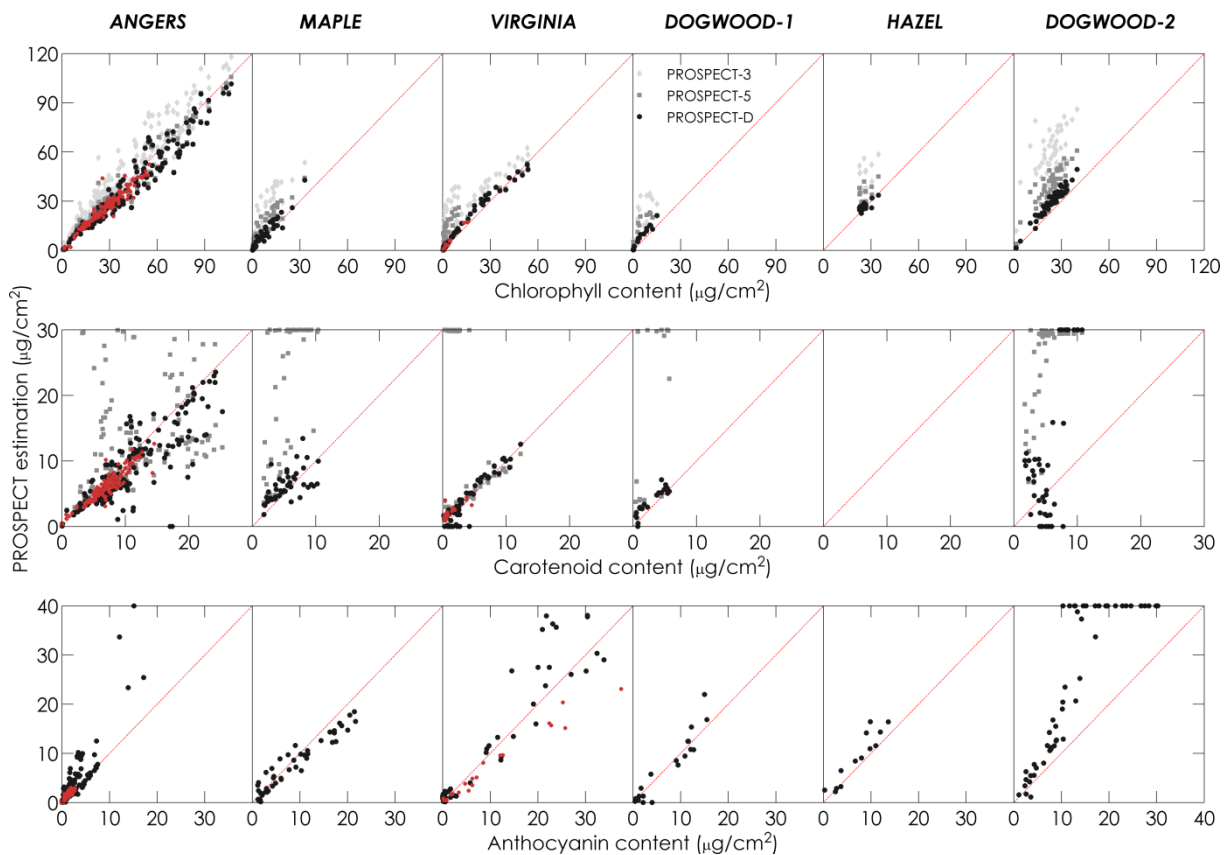


Figure 6. Estimation of pigment content by inversion of three versions of PROSPECT on six datasets

(when relevant). Red dots correspond to calibration samples from ANGERS and VIRGINIA.

453

454 4.4.b. Spectrum reconstruction

455 We compared the spectral RMSE between measured spectra and spectra reconstructed by the
 456 last three versions of PROSPECT after model inversion on the VALIDATION dataset (Figure 7). Values
 457 obtained with PROSPECT-3 ranged between 2% and 6% over the VIS. This model uses a unique SAC to
 458 account for total pigment absorption; therefore it solely applies to healthy green leaves. The
 459 dissociation of chlorophylls from carotenoids in PROSPECT-5 explains the strong decrease in RMSE
 460 between 400 nm and 500 nm where carotenoids absorb light. However, the discrepancies are still
 461 strong between 500 nm and 600 nm where anthocyanins absorb light, and between 650 nm and 750
 462 nm, a spectral domain that corresponds to the second absorption peak of chlorophylls. Finally,
 463 PROSPECT-D surpassed the previous versions with RMSE ranging between 1% and 2% over the entire
 464 VIS.

465

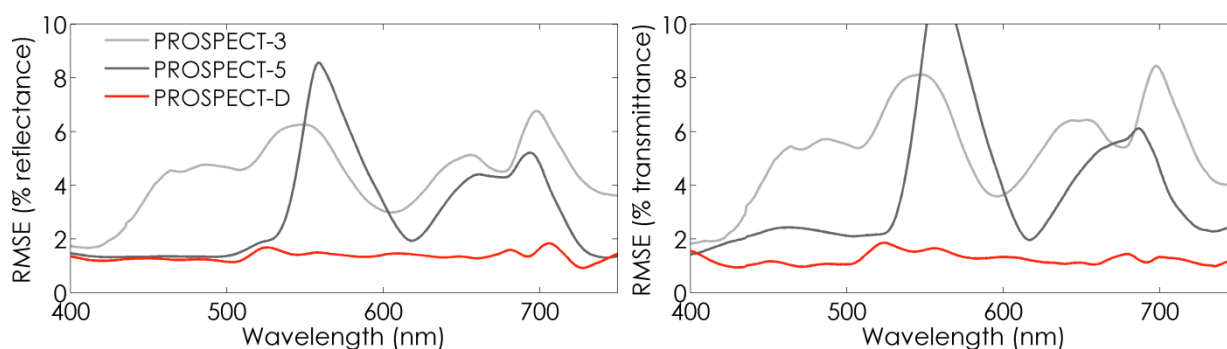


Figure 7. Spectral RMSE between measured and estimated leaf reflectance and transmittance obtained for the VALIDATION dataset after model inversion using PROSPECT-3, PROSPECT-5, and PROSPECT-D.

466

467

468

469 **4.5. Sensitivity to the uncertainty associated with C_{anth} in ANGERS**

470 4.5.a. Specific absorption coefficients

471 The addition of noise to C_{anth} in ANGERS influences the calibration of the SACs of PROSPECT, as
 472 expected, but the variability is limited to the 400-500 nm wavelength range, especially for
 473 anthocyanins. The main difference for this pigment is observed between 500 nm to 600 nm (Figure
 474 8).

475

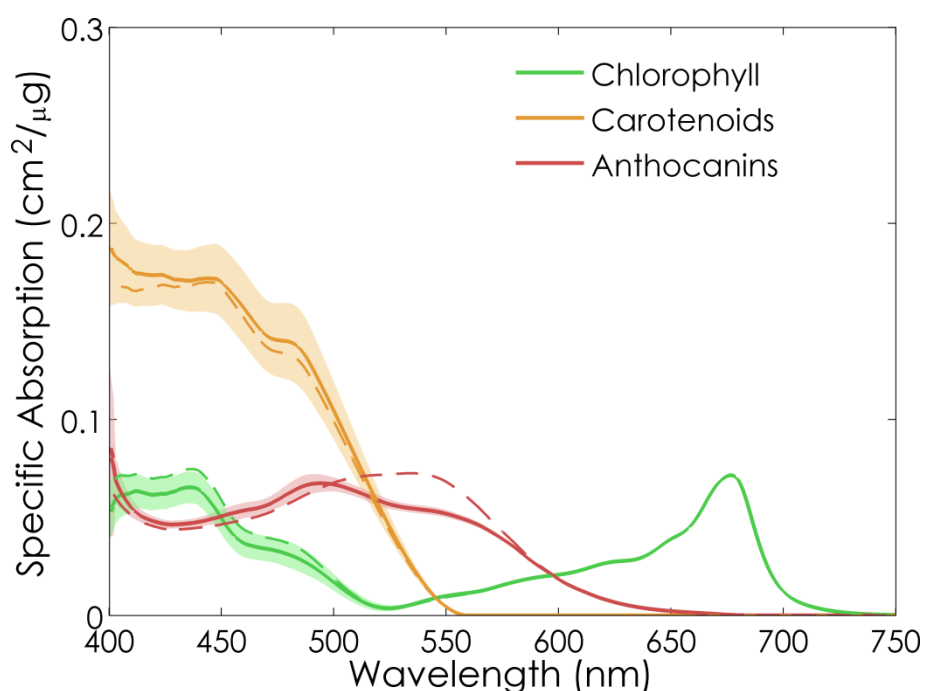


Figure 8. Comparison of the SACs obtained for the three pigments when using C_{anth} values directly derived from *mARI* (dashed lines) and when using C_{anth} values with noise added corresponding to the error of prediction of C_{anth} observed for experimental data (50 repetitions; plain lines and their envelope correspond to mean value \pm 1 standard deviation).

476

477 4.5.b. Estimation of pigment content

478 We estimated pigment content by inverting PROSPECT with the set of SACs derived from noisy C_{anth} .

479 **Erreur ! Source du renvoi introuvable.** summarizes the distributions of RMSE from measured and

480 estimated pigment contents for the validation datasets taken separately and for the VALIDATION
 481 dataset that group them together. The uncertainty on C_{anth} has no influence on the estimation of
 482 C_{ab} , as the RMSE is close to that obtained when no noise was added to C_{anth} . The influence is higher
 483 on the estimation of carotenoids. The version of the model with no noise added to C_{anth}
 484 outperformed the versions including noise, particularly for MAPLE. This version also outperformed all
 485 noisy versions when focusing on VALIDATION. Finally, the estimation of C_{anth} also performed better
 486 when no noise was added to the ANGERS calibration samples, except for MAPLE, which performs a
 487 little less now. However as for C_{xc} , the results obtained with VALIDATION showed better estimation
 488 of C_{anth} when no noise was added. These results validate the calibration of PROSPECT-D with C_{anth}
 489 determined using a spectral index. Moreover, the slight decrease in performances for the estimation
 490 of C_{anth} and C_{xc} when adding noise to C_{anth} suggests that the estimated values obtained for C_{anth}
 491 in ANGERS may show lower uncertainty than expected based on the experimental data available.
 492

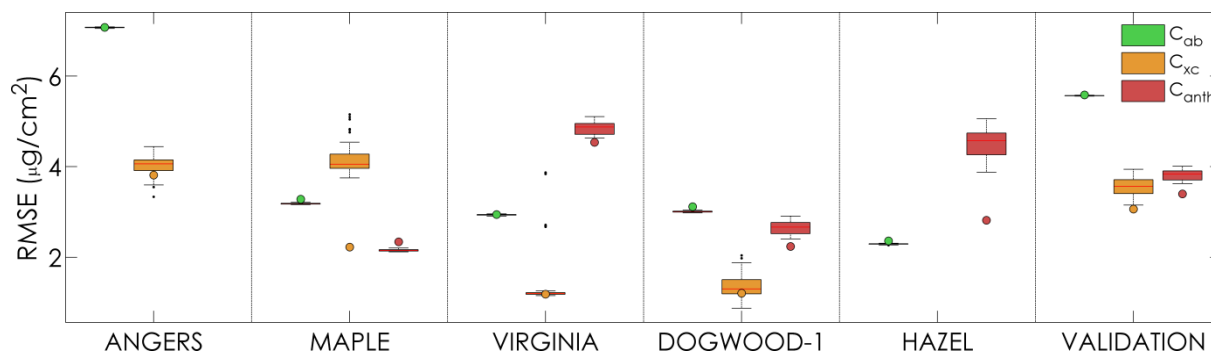


Figure 9. Distribution of the RMSE between measured and estimated pigment content after adding noise to the values of C_{anth} of ANGERS used for calibration of PROSPECT and inversion of the model based on the SACs displayed in Figure 8. Colored dots correspond to the RMSE obtained when no noise is added to C_{anth} in ANGERS.

493

494 4.5.c. Simulation of leaf optical properties

495 The accuracy of reflectance and transmittance reconstruction is displayed in Figure 10. The spectra
 496 simulated by PROSPECT-D show very minor differences whatever noise was added to C_{anth} or not.
 497 These results confirm that despite missing values and indirect estimation of C_{anth} for ANGERS,
 498 PROSPECT-D provides stable results in terms of calibrated SACs as well as model performance both in
 499 direct and inverse modes.

500

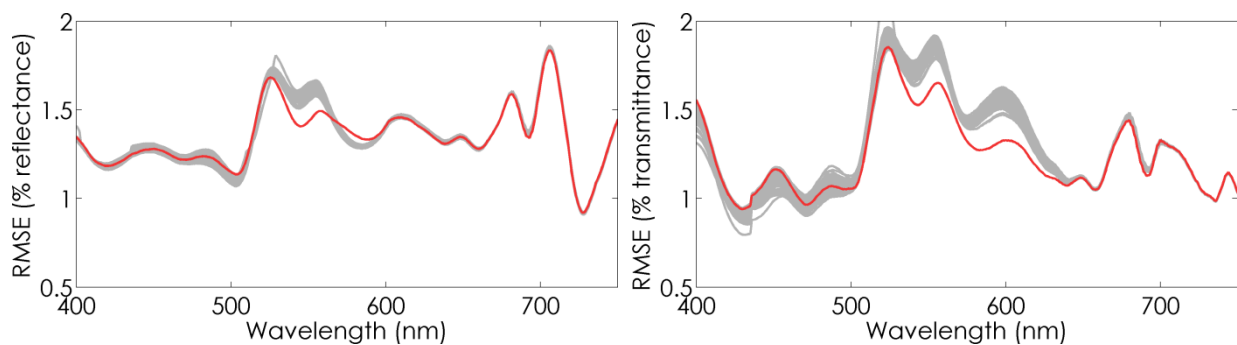


Figure 10. Spectral RMSE between measured and estimated leaf reflectance and transmittance obtained for the VALIDATION dataset after model inversion using PROSPECT-D calibrated with (grey lines) and without (red lines) uncertainty added to C_{anth} .

501

502 5. Discussion

503 5.1. Specific absorption coefficients

504 As stated earlier, the SAC of anthocyanins obtained after the calibration phase (Figure 5)
 505 displayed a broad absorption peak centered on 550 nm. The overall profile agrees well with the
 506 spectra obtained in previous studies (Gitelson et al., 2001; Peters and Noble, 2014). We compared
 507 this SAC with absorption spectra of pure cyanidin-3-glucoside (C3G), measured in the lab for
 508 different pH (Fossen et al., 1998). C3G is also the most common anthocyanin in leaves (Harborne and
 509 Williams, 1998). The SACs derived from a model generally do not match data published in the
 510 literature for *in vitro* dissolved pigments. Several causes may explain such a discrepancy. First,
 511 radiative transfer model are imperfect: for instance, a two-layer model taking into account the

512 asymmetry of leaf anatomy and chemical content in Dicots may improve the determination of the
513 SACs. Moreover, the calibration of these models is based on experimental data which may be not
514 optimal in terms of sampling, despite our efforts to provide the best experimental datasets. Second,
515 molecules *in vivo* are linked to their environment, which may induce shifts in their SACs: this is the
516 case for chlorophylls with proteins, or anthocyanins with the pH or temperature (Figueiredo et al.,
517 1999; Yabuya et al., 1999; Boulton, 2001). Thirdly, while the model assumes stable compositions and
518 absorption spectra for chlorophylls, carotenoids and anthocyanins, in reality these are families of
519 pigments containing between two and hundreds of molecules, with as many variations in their
520 particular absorption spectra. Subtle changes in anthocyanin composition, chlorophyll *a:b* ratio, or
521 induced by the xanthophyll cycle may result in variations in leaf reflectance and transmittance that
522 are detectable but not interpretable by PROSPECT. Finally, as explained earlier, internal multiple
523 scattering (detour effect) and distributional errors (sieve effect) may also explain part of the
524 discrepancies observed between *in vivo* and *in vitro* SACs. **Figure 11** shows the profile of the SAC of
525 anthocyanins: it is intermediate between the absorption coefficient measured for C3G at pH 1 and
526 pH 8.1 in the main absorption domain of this constituent. The increasing absorption closer to the UV-
527 A may be explained by the presence of flavonols in some leaves: these molecules, which are
528 biosynthetically associated with anthocyanins in plant secondary metabolism, are also optically
529 active in this domain. Therefore, it is likely that flavonols contribute to the anthocyanin SAC spectrum
530 (Solovchenko et al., 2001). The improvement of C_{xc} estimation accuracy upon incorporation of
531 anthocyanins into PROSPECT-D may stem from the inherent correlation between anthocyanin and
532 flavonoid content.

533

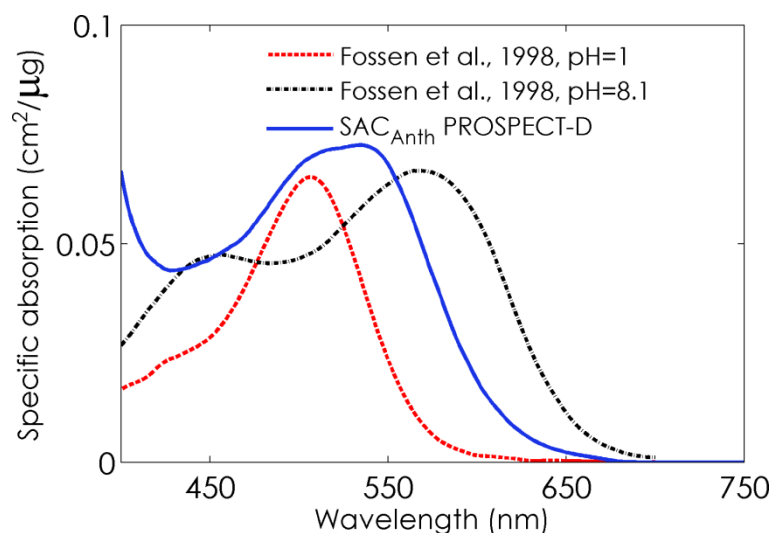


Figure 11. Comparison of the SAC corresponding to anthocyanins derived from PROSPECT-D calibration ($\text{cm}^2 \mu\text{g}^{-1}$) and the absorption of pure Cyanidin 3-glucoside at pH 1 and 8.1 (unitless) (after [Fossen et al., 1998](#)).

534

535 The SAC of carotenoids above 450 nm is very similar in PROSPECT-5 and PROSPECT-D. However
 536 the estimation of C_{xc} is substantially improved in the latter, which highlights the high sensitivity of
 537 PROSPECT to very small changes of the SAC, as well as the importance of incorporating anthocyanins
 538 into the model even for leaves with low content. This improvement in the estimation of C_{xc} was not
 539 explained by the differences observed between 400 nm and 450nm: when using all the ANGERS
 540 dataset for calibration, the SAC calibrated for C_{xc} showed very similar profile as in PROSPECT-5, but
 541 the improvement in the estimation of C_{xc} was still observed.

542

543 5.2. Illustration of the improved simulation of leaf optical properties

544 To illustrate the ability of PROSPECT-D to simulate leaf optical properties we selected some
 545 samples including senescent and reddish leaves ([Figure 12](#)). In some cases, the fit is poor: the
 546 reflectance spectrum of *Eucalyptus gunnii* is very high compared to that of other leaves, probably
 547 due to the presence of wax at the leaf surface, a layer that is not accounted for in PROSPECT ([Figure](#)

548 12h). Barry and Newnham (2012) already pointed out an incorrect assessment of carotenoid content
 549 in *Eucalyptus globulus* and *Eucalyptus nitens* leaves. The development of PROSPECT-D partly
 550 answers these limitations, but further efforts will be needed to include specular reflection (Bousquet
 551 et al., 2005; Comar et al., 2014; Jay et al., 2016).
 552

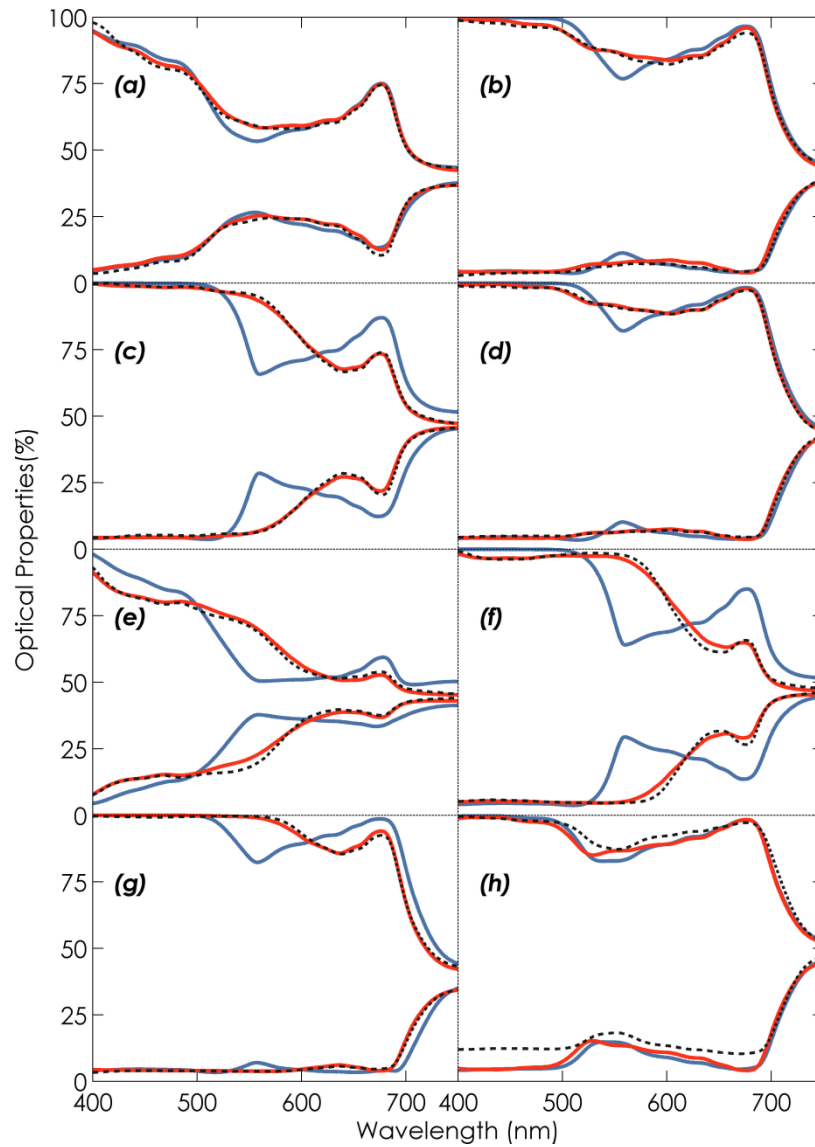


Figure 12. Measured (black dotted line) versus simulated (blue line for PROSPECT-5 and red line for PROSPECT-D) reflectance (lower spectra) and transmittance (upper spectra). (a-b) *Acer pseudoplatanus* L., (c) *Acer platanoides* L., (d) *Corylus avellana* L., (e-f) *Parthenocissus quinquefolia* (L.) Planch., (g) *Corylus maxima* 'Purpurea', and (h) *Eucalyptus gunnii*. Samples (b-e-f) show non-

senescent anthocyanic leaves, while samples (a-c-d-g) show anthocyanic senescent leaves.

553

554 **6. Conclusion**

555 We introduced a new, physically-based model called PROSPECT-D which, for the first time,
556 includes the three main families of leaf pigments as independent constituents: chlorophylls,
557 carotenoids, and anthocyanins. PROSPECT-D outperformed the previous versions of the model, both
558 for the estimation of leaf chemical constituents and the simulation of leaf optical properties, on
559 datasets encompassing a broad range of growth conditions and stages. Inversion of PROSPECT-D
560 showed improved estimation of pigment content, especially carotenoid content. These results
561 demonstrate the ability of this new model to simulate optical properties during the lifespan of the
562 leaf and for a new range of conditions, including juvenile and senescent stages, as well as
563 environmental stresses.

564 We studied the influence of the uncertainty corresponding to the values of C_{anth} from
565 ANGERS used for the calibration of PROSPECT-D. Our results showed that this uncertainty has little to
566 no impact on the calibration, and on the performances of the model in terms of pigment prediction
567 accuracy, as well as in modeling of leaf optics.

568 The availability of this model has strong implications for vegetation modeling both at leaf and
569 canopy scales. At the leaf scale, PROSPECT-D will allow to perform sensitivity analyses focused on
570 anthocyanins, and to design new vegetation indices dedicated to specific pigments and less sensitive
571 to other ones. The linkage of PROSPECT-D with canopy reflectance models such as SAIL (e.g.,
572 Jacquemoud et al., 2009) or DART (Hernández-Clemente et al., 2012; Gastellu-Etchegorry et al.,
573 2015) will allow simulations of vegetation types that could not be reproduced before. Applications
574 for stress and senescence detection will directly take advantage of such improvements. Finally
575 PROSPECT-D is a powerful tool for determining the potential of operational multispectral satellites
576 (Sentinel-2, LandSat-8, WorldView-3) and future hyperspectral missions (EnMAP, Hypispi, HYPXIM)
577 for fine detection of leaf pigments.

578

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591

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- 817

818 **Figure 1.** Relationship between C_{anth} obtained from wet chemistry and C_{anth} estimated from
 819 reflectance data after application of Eq. 2. The black dots correspond to the 137 leaf samples with
 820 $mARI < 5$ ($R^2 = 0.90$) and the grey dots correspond to the 76 leaf samples with $mARI > 5$ ($R^2 =$
 821 0.37). Eq. 2 was adjusted only on the black dots.

822

823 **Figure 2.** Comparison of the refractive index used in PROSPECT-3 (red dots), PROSPECT-5 (grey
 824 diamonds) and corresponding to the mean refractive index proposed by (Chen and Weng, 2012)
 825 (blue squares). The grey area corresponds to the range of variation of the refractive index proposed
 826 by Chen and Weng, (2012); the plain grey line corresponds to the refractive index for pure liquid
 827 water (Hale and Querry, 1973).

828

829 **Figure 3.** Stacked distribution of pigment content for the calibration and validation samples selected
 830 from ANGERS and VIRGINIA. Light colors: calibration (144 samples from ANGERS, 20 Samples from
 831 VIRGINIA); dark colors: validation (164 samples from ANGERS, 61 samples from VIRGINIA).

832

833 **Figure 4.** Spectral RMSE between measured leaf optics and simulated reflectance (left), and
 834 transmittance (right) obtained after model inversion with the refractive index used in PROSPECT-5
 835 (black dotted line), PROSPECT-3 (red), and derived from Chen and Weng (2012) (light grey).

836

837 **Figure 5.** Specific absorption coefficients of chlorophylls (green), carotenoids (orange), and
 838 anthocyanins (red). The solids line correspond to the SAC of all the pigments in PROSPECT-D, the
 839 dashed lines to the SAC of chlorophylls and carotenoids in PROSPECT-5, and to the SAC of
 840 anthocyanins measured by Peters and Noble (2014) using thin layer chromatography.

841

842 **Figure 6.** Estimation of pigment content by inversion of three versions of PROSPECT on six datasets
843 (when relevant). Red dots correspond to calibration samples from ANGERS and VIRGINIA.

844

845 **Figure 7.** Spectral RMSE between measured and estimated leaf reflectance and transmittance
846 obtained for the VALIDATION dataset after model inversion using PROSPECT-3, PROSPECT-5, and
847 PROSPECT-D.

848

849 **Figure 8.** Comparison of the SACs obtained for the three pigments when using C_{anth} values directly
850 derived from $mARI$ (dashed lines) and when using C_{anth} values with noise added corresponding to
851 the error of prediction of C_{anth} observed for experimental data (50 repetitions; plain lines and their
852 envelope correspond to mean value ± 1 standard deviation).

853

854 **Figure 9.** Distribution of the RMSE between measured and estimated pigment content after adding
855 noise to the values of C_{anth} of ANGERS used for calibration of PROSPECT and inversion of the model
856 based on the SACs displayed in Figure 8. Colored dots correspond to the RMSE obtained when no
857 noise is added to C_{anth} in ANGERS.

858

859 **Figure 10.** Spectral RMSE between measured and estimated leaf reflectance and transmittance
860 obtained for the VALIDATION dataset after model inversion using PROSPECT-D calibrated with (grey
861 lines) and without (red lines) uncertainty added to C_{anth} .

862

863 **Figure 11.** Comparison of the SAC corresponding to anthocyanins derived from PROSPECT-D
864 calibration ($\text{cm}^2 \mu\text{g}^{-1}$) and the absorption of pure Cyanidin 3-glucoside at pH 1 and 8.1 (unitless) (after
865 [Fossen et al., 1998](#)).

866

867 **Figure 12.** Measured (black dotted line) versus simulated (blue line for PROSPECT-5 and red line for
868 PROSPECT-D) reflectance (lower spectra) and transmittance (upper spectra). (a-b) *Acer*
869 *pseudoplatanus* L., (c) *Acer platanoides* L., (d) *Corylus avellana* L., (e-f) *Parthenocissus quinquefolia*
870 (L.) Planch., (g) *Corylus maxima 'Purpurea'*, and (h) *Eucalyptus gunnii*. Samples (b-e-f) show non-
871 senescent anthocyanic leaves, while samples (a-c-d-g) show anthocyanic senescent leaves.

872

873 **Table 1.** Description of the leaf datasets used in this study.

874

875 **Table 2.** RMSE ($\mu\text{g cm}^{-2}$) of the estimation of leaf pigment content using PROSPECT-3 (P-3),
876 PROSPECT-5 (P-5), and PROSPECT-D (P-D) inversion. The bold font for numbers indicates the lowest
877 values.

878