Sensitive detection of chloroplast movements through changes in leaf cross-polarized reflectance

Running title: Chloroplast movement detection from polarized reflectance

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Abstract

We present a sensitive method for non-contact detection of chloroplast movements in leaves and other photosynthetic tissues, based on changes in the magnitude of cross-polarized reflectance of the leaf. We created a custom optical set-up and dedicated software for measurements of changes in red light reflectance during irradiation with blue light, known to trigger chloroplast relocations. Experiments on *Arabidopsis thaliana* wild type and several mutants with disrupted chloroplast movements showed that the chloroplast avoidance response, induced by high blue light, led to a substantial increase in diffuse reflectance of unpolarized red light. The effects of the accumulation response in low blue light were the opposite. The specular reflectance of the leaf was unaffected by the chloroplast positioning. To further improve the specificity of the detection, we examined the effects of chloroplast relocations on the leaf reflectance of a linearly polarized incident beam. The greatest relative

change associated with chloroplast movements was observed when the planes of polarization of the incident and detected beams were perpendicular. This suggests that the chloroplast positioning affects the magnitude of depolarization of light. We applied the developed approach to examine chloroplast relocations in four angiosperm species collected in the field. In particular, the method allowed us to detect the chloroplast avoidance response in the green stems of bilberry before bud break. Such samples are not amenable to transmittance-based detection.

Significance statement

Despite the importance of chloroplast movements for the optimization of photosynthetic efficiency and biomass production, high throughput reflectance-based methods are not routinely used for their detection. Strong dependence of the amount of specularly reflected light reaching the detector on the leaf orientation may lead to a substantial error in chloroplast relocation measurements. We show that the assessment of chloroplast responses through polarized reflectance reduces systematic errors due to the leaf movements and improves the sensitivity of detection. This opens a possibility of non-invasive, non-contact detection of chloroplast relocations using existing instrumentation for remote sensing of agriculturally relevant crop traits.

Introduction

Chloroplast movements allow plants to achieve high photosynthetic performance and biomass production in dynamic light conditions (Hart et al. 2019). In low light, chloroplasts gather at cell walls perpendicular to the light direction to maximize photosynthetic efficiency. In high light, chloroplasts move to cell walls parallel to the light direction as a photoprotective mechanism. In flowering plants, chloroplast relocations are triggered by blue light, perceived through UV/blue light receptors phototropins (Banaś et al., 2012), (Łabuz et al., 2022). Two phototropins control chloroplast movements in the model plant *Arabidopsis thaliana*. Phot1 and phot2 trigger chloroplast accumulation (Sakai et al., 2001), while sustained chloroplast avoidance is elicited only by phot2 (Jarillo et al., 2001), (Kagawa et al., 2001). Chloroplast relocations cause substantial changes in the optical properties of the leaf, affecting its transmittance and reflectance (Zurzycki 1961). As red light usually does not induce the movements in angiosperms, it is used to monitor changes in leaf optical properties triggered by actinic blue irradiation. The most widely used approach to the detection of chloroplast relocations consists in the recording of light-induced transmittance changes, both in the

laboratory (*e.g.* Inoue and Shibata 1973) and in field conditions (Williams et al. 2003). The transmittance-based detection is robust and repeatable provided that an integrating sphere or the opal method (Shibata et al 1954, Walczak and Gabryś 1980) is employed to eliminate the dependence of the signal on the sample-detector distance. Transmittance-based methods require direct access to the leaf, which limits their throughput and applicability in field conditions. An alternative approach is to detect chloroplast relocations through measurements of the changes in leaf reflectance. An increase in leaf reflectance accompanying the avoidance response has been first observed in *Begonia multiflora* (Seybold 1956) and *Lemna trisulca* (Zurzycki 1961). The detection of chloroplast movements through changes in white or red light leaf reflectance has been reported by (Park et al., 1996, Davis et al., 2011, Baránková et al., 2016, Dutta et al., 2015, Dutta et al., 2017).

An important drawback of reflectance-based measurements of plant traits is the strong dependence of the amount of reflected light reaching the detector on the orientation of the leaf (Li et al 2018). Specular reflectance exhibits strong angular dependence, hindering chloroplast movement detection, as reported in *Alocasia macrorrhiza* (Gorton et al., 1999). Several potential approaches can be proposed to alleviate the effect of the leaf orientation on the reflectance-based chloroplast movement detection, which may be used in concert: 1) simultaneous detection of reflectance at several angles of observation, 2) measurement of the plant/canopy geometry in parallel to reflectance measurement, 3) incorporation of polarization information. This study aimed to determine whether the specificity of chloroplast movement detection can be improved by analyzing the polarization of light reflected from the leaf. Unpolarized light specularly reflected by the wax layer or the cuticle becomes partially polarized, with the degree and the plane of polarization dependent on the orientation of the leaf. By contrast, light diffusely reflected from the leaf interior, through multiple refractions at the border between intercellular spaces and mesophyll cell walls, does not undergo substantial polarization (Vanderbilt et al. 1985). This suggests that by separating the polarized and non-polarized components of the reflected light, the detection of chloroplast movements independently of the spatial orientation of the leaf would be feasible. The diffuse and specular reflection mechanisms also differ in their ability to depolarize light. When the leaf is illuminated with a polarized beam, light reflected from the leaf surface does not undergo depolarization. By contrast, the diffuse reflectance from the leaf interior undergoes substantial depolarization (Rvachev and Guminetskii 1966, Brakke 1994), likely due to multiple scattering. This suggests that the cross-polarized reflectance may contain information about

the state of the leaf mesophyll, in particular, the positioning of chloroplasts. Previously, the magnitude of depolarization was used to remotely sense the nitrogen status of crops (Kalshoven et al. 1995), possibly due to the nitrogen-availability effects on the chlorophyll amount.

To examine the link between chloroplast positioning and polarized red light reflectance, we used the *Arabidopsis thaliana*, wild-type Columbia and photoreceptor mutants, *phot1, phot2* (lacking sustained chloroplast avoidance), and *phot1phot2* (disrupted chloroplast movements). We also employed a signaling mutant, *jac1,* deficient in chloroplast accumulation (Suetsugu et al., 2005). These mutants were in the Columbia background, with leaves covered with trichomes. To investigate whether the presence of trichomes affects the performance of the reflectance-based detection of chloroplast relocations, we also examined the *glabra1* mutant. To assess the extent of applicability of the developed methods, we have examined their performance in photosynthetic organs of several wild species.

Materials and Methods

Plant material

WT Col-0 *Arabidopsis*, *glabra1, phot1* (SALK_088841) (Lehmann et al., 2011), *jac1-3* (WiscDsLox457-460P9) (Hermanowicz et al., 2019), *phot2* (Jarillo et al., 2001), *phot1phot2* (Hermanowicz et al., 2019), were sown in Jiffy-7 pots (Jiffy Products International AS) and stratified at 4°C for 2 days. Plants were cultivated in a growth chamber (Sanyo MLR 350H) at 23°C, 80% relative humidity, a photoperiod of 10 h light and 14 h darkness, at photon irradiance of 70 (±15) $\mu mol \cdot m^{-2} \cdot s^{-1}$ (measured at the plane parallel to the rosettes) supplied by fluorescent lamps. The position of plants from different lines in the chamber was randomized and the trays with plants were rotated 6 – 7 times per week. The experiments were performed on rosette leaves detached from dark-adapted 5-week-old plants.

Wild plants were collected in March 2023. Plants were harvested in the afternoon, kept dark-adapted in water, and measured the next day. Flowering specimens of *Draba verna* (common whitlowgrass) were collected in a xeric meadow covering the south-facing slope of Górka Pychowicka hill (Kraków, 50°1'45" N 19°53'6" E). Chloroplast movements were induced by irradiation of the adaxial side of rosette leaves (Fig. 4 B). *Stellaria media* (chickweed) was collected in a young birch-alder forest near Jagiellonian University Campus in Kraków, Poland (50°1′44″ N 19°54'6" E). The adaxial surface of the third leaf from the top of the plant (asterisk in Fig. 4 C) was irradiated. *Tussilago farfara* (coltsfoot) was collected at the human-disturbed sites (lawns and construction sites) near the Jagiellonian University Campus.

Chloroplast movements were induced by irradiation of the abaxial (sun-exposed) side of the scales covering flowering shoots (Fig. 4 D). *Vaccinium myrtillus* (bilberry) stems were collected in a fir forest on the north slope of Mnisko Mt in the Gorce Range, Poland (750 m a.s.l, 49°34'40" N 20°2'39"). Chloroplast movements were examined in terminal branches (asterisk in Fig. 4 E).

Definitions and conventions

We will use the term bidirectional reflectance $R(\theta_i,\varphi_i,\theta_v,\varphi_v,)$ to refer to the ratio between radiance L of the sample when viewed along the direction (θ_v,φ_v), and the E irradiance received from the direction (θ_i,φ_i) (Nicodemus 1965, Schaepman-Strub et al. 2006), where θ and φ are polar and azimuthal angles, respectively. As in Vanderbilt et al. (1985), we calculate polarized reflectance as the ratio of the polarized reflectance and part of the irradiance corresponding to the appropriate polarization state, even when the incident radiation was unpolarized. Light polarized in the plane of the normal and the incident ray is referred to as *P*-polarized. Light polarized in the plane perpendicular to the plane of incidence is described as *S*-polarized. In our experiments, the incident rays, the leaf normal and the direction of observation were all contained in the same plane. For simplicity, we specify the directions of incidence and observation using a single angle, measured with respect to the leaf normal, as shown in Fig. 1 A. In this convention, a positive angle α corresponds to the polar angle α and azimuth of 0°, while a negative angle -α describes the same direction as the polar angle α and azimuth of 180°.

Optomechanical setup for measurement of blue light-induced changes in leaf bidirectional reflectance

The measuring beam was produced by a red LED (nominal wavelength of 660 nm, M660L4, Thorlabs) and collimated with an achromatic lens (#49778, Edmund Optics). The collimated beam was passed through a bandpass interference filter (660 nm max, 10 nm FWHM, #15-126, Edmund Optics) to reduce the width of its spectrum. In some experiments (Fig. 3-4), a linear polarizer (#47316, Edmund Optics) was inserted next to the filter. The actinic beam was supplied by a blue LED (nominal wavelength of 455 nm, M455L4, Thorlabs), collimated with an aspherized achromatic lens (#49-662, Edmund Optics), and passed through a bandpass interference filter (450 nm, 25 nm FWHM, #15146, Edmund Optics). The spectra of the beams measured at the leaf mount are shown in Fig. S1. The beams were combined with a dichroic mirror (DMLP550, Thorlabs) and passed through a condenser composed of two achromatic lenses (AC 254-040-A, Thorlabs) and an iris diaphragm. The condenser was

designed to ensure uniform illumination at the leaf plane. The setup also included field diaphragms to control the size of the red and blue beams. The radius of the red beam at the sample plane was set to 1.5 mm, to avoid illuminating the midvein, while its power was ca. 29 µW (when unpolarized) or ca. 13 µW (when polarized). The radius of the blue beam was 6 mm. The field of view of the detector (ca. 12 mm in radius) exceeded the size of the red spot, thus, the detected signal was proportional to radiant intensity, and was divided by the cosine of the observation angle to obtain radiance. The leaf was placed in a custom-made holder (Fig. 1B) mounted in a gimbal (GMB1, Thorlabs), which allowed for control of the angle between the leaf surface and the direction of incident light. The holder consisted of two parts, both with an ellipsoidal hole in the middle, between which the leaf fragment was inserted. Edges of the specimen were covered with a piece of wet tissue paper. The angle between the incident beam and the leaf normal was kept at 40° or 55°. The angle of 55° corresponds to Brewster's angle for the interface between air and a sample made of material with a refractive index n of 1.43, close to the values reported for epicuticular waves (Jacquemoud and Ustin 2019, p. 72). When the angle of incidence is equal to Brewster's angle and the sample is flat, the specularly reflected ray is completely polarized, in the *S* plane, even when the incident beam is unpolarized. The reflected light was collected at the angle of observation of 0° (diffuse reflection) and either -40° or -55° (diffuse and specular reflection) with respect to the leaf normal. It passed through a linear polarizer (#47316, Edmund Optics) and a bandpass interference filter (660 nm, #15-126, Edmund Optics) blocking actinic radiation and most of the chlorophyll fluorescence. The filtered light was focused on a photodetector (amplified silicon photodiode, PDA100A2, Thorlabs) with a plano-convex lens (LA1074-A, Thorlabs). The angular size of the clear aperture of the collecting lens with respect to the sample center was 0.019 steradian (calculated using our ray-tracing Mathematica package https://github.com/plantPhotobiologyLab/openRayTracer). To control the observation angle, the detector was mounted at the RBB300A/M rotation board (Thorlabs). The experiments were performed with two angular positions of the polarizer: its transmission axis was either parallel (transmits *P*) or perpendicular (transmits *S* component) to the plane of incidence. The LEDs supplying the measuring and the actinic beams were controlled with two Cyclops drivers (Newman et al 2015). The measuring beam was amplitude-modulated at 1013 Hz. The sinusoidal signal was generated with a programmable FPGA board (STEMLab 125-14, Red Pitaya, Slovenia) and fed to a Cyclops driver. Modulation of the measuring beam allows for the reduction of detector noise when the lock-in detection method is used. The signal from the detector was fed into the Red Pitaya board,

running a lock-in amplifier (Luda et al 2019). The actinic beam driver was controlled from a PC, through a serial connection with the onboard Teensy 3.2 microcomputer. On the PC side, the experimental setup was controlled using BeamJ, our publicly available Java application (https://github.com/pawelHerm/beamJ). The application was designed for the control of custom optomechanical systems used for the analysis of light-induced changes in the optical properties of the leaves. A silver mirror (#49194, Edmund Optics) placed in the gimbal was used to record the detected signal produced when the radiant flux incident on the sample was completely reflected. A white, diffusely reflecting PTFE disk (SM05CP2C, Thorlabs) was also measured whenever the setup was calibrated. However, the reported values of bidirectional reflectance are absolute (not divided by the PTFE signal). Axes of polarizers were determined using the two-polarizer method of Rowell et al. (1970). The axis of the analyzer was additionally checked using light that underwent *S*-polarization upon reflection from a glass window (#83375, Edmund Optics), placed in the sample gimbal, and illuminated with the measuring beam at Brewster's angle. Leaf reflectance curves were processed with a custom-written Mathematica (Wolfram Research, USA) script.

Measurements of blue-light-induced changes in leaf hemispherical transmittance

Hemispherical leaf transmittance changes due to chloroplast movements were assessed with the photometric method (Walczak and Gabryś 1980) (Gabryś et al., 2017), using a custom-built photometric setup. The device used the same blue and red LED sources as in the reflectometric setup The detached leaf was mounted in front of a port of an integrating sphere (IS200-4, Thorlabs). The signal was detected with a photodiode detector (DET100A2, Thorlabs), which was mounted at another port of the sphere. Light reaching the detector was filtered with an absorptive/interference compound filter (660 nm max, BN660, Midwest Optical Systems, USA) to reduce shifts in the transmission band of an interference filter.

Light measurements

Chloroplast movements were induced with either 0.1 µmol m^{-2} s^{-1} (accumulation) or 100 µmol m^{-2} s^{-1} (avoidance) of blue light. The spectra of actinic and measuring light used in the reflectometric setup were measured with a BLACK Comet SR spectrometer (25 nm slit, Stellar Net, USA), equipped with a cosine corrector. The irradiance of blue light was measured at the sample plane with the LI-190R sensor (LI-COR, USA), combined with the Keithley 6485 picoammeter. Due to the small size of the spot of the red beam at the sample plane in the reflectometric setup, its power was measured with the S170C microscope slide power sensor (Thorlabs).

Confocal microscopy

Microscopic observations were performed with the Axio Observer.Z1 inverted microscope (Carl Zeiss, Germany) and the LSM 880 confocal module. The long-distance LCI Plan-Apochromat 25×, NA 0.8, objective was used with glycerol immersion. Chlorophyll was excited with the 633 nm He–Ne laser, fluorescence emission was recorded in the range of 659–721 nm. Chloroplast positioning was observed in the parenchyma of plant leaves or stems irradiated in the reflectometer setup for 1 h. Z-stacks were collected on the adaxial surface of *Draba verna* and *Stellaria media* leaves, the abaxial side of *Tussilago farfara* scale leaves and *Vaccinium myrtillus* stems, with the interval between slices set to ca. 2.4 μm. Projection images were calculated from slices spanning the epidermis and palisade layer (60 - 120 μm of tissue, depending on the species), starting from the upper surface of the epidermis.

Statistical analysis

The observed distributions of bidirectional reflectance changes substantially deviate from normality when the detector is positioned in such a way as to collect specularly reflected light. The distributions are heavy-tailed, with an excess of extreme values as compared to the normal distribution. The specular lobe of the leaf reflectance function is relatively sharp, so even relatively small changes in leaf undulations during measurement (*e.g.* induced by the actinic light) may cause part of the specularly reflected beam to miss the detector. In addition, when specularly reflected light is collected, the observed variance depends on the orientation of the polarizer, thus, the homoskedasticity assumption of the standard ANOVA is not satisfied. To reduce the effect of extreme observations, we have transformed the amplitudes and rates of reflectance changes using the inverse hyperbolic sine transformation of the form $\sinh^{-1}(\theta x)/\theta$, where x is the untransformed value of the response variable, while θ is a parameter that needs to be estimated. The linear model of the effect of the plant line, polarizer orientation, and their interaction on the transformed change of reflectance due to actinic light was fitted using the maximum log-likelihood estimator of the *gls* procedure of the *nlme* package (Pinheiro et al. 2019) in the R software (R Core Team 2019). The model was extended to allow for heteroskedasticity using the variance function of the form *varIdent(form = ~1|Polarizer)*. The parameter θ of the transformation was found through maximization of the concentrated log-likelihood (Burbidge 1988), calculated as the sum of the logarithm of the determinant of the jacobian of the transformation and the maximum log-likelihood values produced by *gls*. The values of θ which gave the greatest concentrated log-likelihood were selected. The significance of differences in means of transformed values between particular

groups was assessed with Tukey's method, using the model fitted by the *gls* procedure as the input for the *emmeans* command (Lenth 2021) and the *cld* command of the *multcomp* package (Hothorn et al. 2008). The amplitudes and rates of hemispherical transmittance changes followed the normal distribution, thus, the statistical analysis was performed on untransformed values. The statistical analysis of transmittance changes was otherwise performed in the same way as for the reflectance measurements.

Results and Discussion

In this work, we built a goniometric setup for monitoring chloroplast movements (Fig. 1). It allowed for the recording of changes in bidirectional leaf reflectance induced by blue light, at adjustable incidence and observation angles. The set-up was equipped with one (on the detector side) or two polarizers (in front of both the detector and red beam source) to reduce the effects of leaf orientation on the detected signal and improve the specificity of chloroplast movement monitoring.

The bidirectional reflectance function of leaves typically features specular lobes (Breece and Holmes 1971), whose prominence depends on the properties of the leaf surface (Bousquet et al., 2005). To examine the effects of orientation of an *Arabidopsis* leaf on its reflectance and ability to polarize light, *P* and *S*-polarized components of light reflected from the dark-adapted leaves were measured at two angles of incidence and several angles of observation (the angle of observation between 0° and -55° for 40° incidence (Fig. S2 A), and the angle between 15° and -55° for 55° incidence (Fig. S2 B)). In those experiments, the setup with one polarizer was used (incident light was unpolarized). In all analyzed *Arabidopsis* lines, radiance strongly depended on the observation angle ($(P + S)/2$ in Fig. S2). The radiance for wild-type leaves in the direction of specular reflection was ca. 2.2 (40°) or 4.8 (55° incidence) greater than the radiance in the direction normal to the leaf surface. A similar dependence of the bidirectional reflectance on the observation angle was observed for other *Arabidopsis* lines. This results demonstrate a considerable magnitude of a spurious signal that could be recorded if chloroplast movements were examined outdoors, *e.g.* in plants exposed to wind. Power of the *S*-polarized component of the reflected light was affected by the angles of incidence and observation to a much greater extent than the power of the *P* component. The estimated degree of linear polarization of reflected light varied strongly with the observation angle (Fig. S2 C), especially when the incidence angle was equal to Brewster's angle. The specular component of leaf bidirectional reflectance depends strongly on the angles of incidence and observation. Leaf movements (*e.g.* nyctinastic or due to wind) will increase

signal variance, but the deviation may be expected to average out when a considerable number of measurements is taken. Systematic errors, however, can arise from light-induced reorientation of leaves and shoots, either photonastic, phototropic or due to wilting. Light-induced changes in leaf orientation have been observed in several plant families. They can be fast and substantial in magnitude. In *Oxalis oregana*, high blue light induces leaflet folding, with the petiole angle decreasing by 90° within a few minutes (Bjorkman and Powless 1981). In *Arabidopsis*, the blade of a leaf changes its orientation within a few hours in response to low blue light (Inoue et al. 2008). Such a rotation of the blade would change the angle of incidence of measuring light, affecting the amount of reflected light received by the detector. Thus, polarization-sensitive detection can improve the reliability of reflectance-based chloroplast movement monitoring in the presence of factors that change the orientation of leaves.

We observed that changes ΔR in leaf reflectance mimic the changes in hemispherical transmittance, monitored in the standard, photometric approach for chloroplast movement detection, in agreement with previous reports (Seybold, 1956, Zurzycki, 1961, Dutta et al., 2015). In low blue light of 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the accumulation response observed in wild-type, *glabra1, phot1,* and *phot2* leaves resulted in a substantial decrease in transmittance (Fig. S3 A, C) and directional reflectance at all combinations of angles of observation and incidence examined in this work (55° incidence shown in Fig. 2 A, B, Fig. S4; 40° in Fig. S5 A, B, Fig. S6). The changes in reflectance were absent in *phot1phot2*, indicating that they were phototropin-dependent. Interestingly, the *jac1* showed a weak, but statistically significant, decrease in transmittance at 0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. S3 A, C). The *jac1* mutant is a signaling mutant, lacking JAC1, the J-domain protein required for chloroplast accumulation response (Suetsugu et al., 2005). It was previously reported that chloroplast accumulation is absent in *jac1*. Instead, this mutant exhibits chloroplast avoidance at a broad range of irradiance values (Suetsugu et al., 2005). Our observations indicate that at very low light irradiances, so far not investigated, chloroplasts may undergo accumulation in this mutant. In high blue light of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the avoidance response observed in wild type, *glabra1*, *phot1,* and *jac1* leaves was accompanied by an increase in transmittance (Fig. S3 B, C) and bidirectional reflectance at both 55° (Fig. 2 C, D, Fig. S7) and 40° (Fig. S5 C, D, Fig. S8) angles of incidence, while the effects of the accumulation response observed in *phot2* had the opposite effect. No significant differences in the magnitude of reflectance changes were observed between wild-type Columbia and *glabra1* leaves, implying that the presence of

trichomes did not exert a substantial effect in *Arabidopsis thaliana* (Fig. 2 A - D). Our finding is consistent with previous results obtained for the *glabra1* mutant showing no significant difference in specular reflectance between this hairless line and the *exo70H4-1* and *arpc5* mutants, both bearing trichomes (Neuwirthová et al. 2021).

The changes in the power of the *P*-polarized component of reflected light were similar in magnitude to changes in the power of the *S*-polarized component and their means were not statistically different. This was the case for all lines both for changes induced with low (Fig. 2A, B, Fig. S5 A, B) and high blue light (Fig. 2 C, D, Fig. S5 C, D). The variance in the observed reflectance changes was smaller when the polarizer transmitted *P*-polarized light (attenuating specular reflection), as compared to the *S*-orientation. As expected, the initial reflectance R_0 of dark-adapted leaves was substantially greater when *S*-polarized light was collected as compared to *P*-polarized light (Fig. S9). Following the approach of Carter (1991) and Baránková et al. (2018), we defined the differential sensitivity of detection as $\Delta R/R_0$ (Fig. S10). Substantial effects of the polarizer orientation were observed when specularly reflected light reached the detector. At Brewster's angle (55°), the sensitivity of detection of the reflectance changes due to chloroplast avoidance in WT was 2.8 times greater when the polarizer was in the *P* orientation as opposed to the *S* orientation (Fig. S10 H). The absence of any substantial effect of the polarizer orientation on raw values of ΔR (Fig. 2, Fig. S5) was consistent with the chloroplast positioning affecting the reflectance component originating from the mesophyll. Although scattering at the cellular components may induce partial polarization, polarization of light reflected from the leaf occurs predominantly at its surface (Shulgin and Moldau 1965, Vanderbilt et al. 1985). Specular reflection arises from the interaction between incoming radiation and the cuticle and it is not affected by the pigment composition of the leaf, as shown in studies on variegated leaves (Grant et al 1987).

To further reduce the specular component and improve the sensitivity of chloroplast movement detection, we inserted an additional polarizer into the path of the measuring beam (Fig. 3). We examined the effects of chloroplast relocations on reflectance for four combinations of orientations of the polarizers: *P*(incident)/*P*(observed); *P*/*S*; *S*/*P*; *S*/*S*. The experiments were performed on the wild-type and the double *phot1phot2* mutant. The angle of incidence was set to 55°, while the angle of observation was either 0° (diffusely reflected light recorded) or -55° (combined specular and diffuse reflectance recorded). The analysis of the chloroplast avoidance response in *Arabidopsis* wild-type plants indicated that the sensitivity of detection $\Delta R/R_0$ was the highest when both polarizers were crossed, i.e. for the

P/*S* and *S*/*P* combinations. In the wild type, the cross-polarized diffuse (0°observation, Fig. 3 A, E, G) reflectance increased two times (mean $\Delta R/R_0 \approx 1$) when leaves were irradiated for 1 h with 100 µmol·m^{-2}·s^{-1} of blue light, pointing to the enhanced depolarization of light in the mesophyll. This effect was absent in *phot1phot2* plants (Fig. 3 C, E, G), indicating that the large increase in depolarization was due to chloroplast movement from dark positioning to avoidance positioning. The small $\Delta R/R_0$ values observed when the detector collected specular reflection and both the incident and observed light was *S*-polarized (Fig. 3 B, F, H) were due to high levels of initial reflectance R_0 (Fig. S11 B), as predicted by Fresnel's equations. The degree of polarization of light reflected from leaves was reported to be inversely correlated with total leaf reflectance. In spectral regions, in which photosynthetic pigments absorb strongly, the less-polarized diffuse component is attenuated through absorption, so that the Fresnel-polarizable specularly reflected light is a major contribution to the total reflected light (Shulgin and Moldau 1965, Wu 2013).

We then investigated the sensitivity of detection based on cross-polarized (*P* incident, *S* analyzed, 0° observation) reflectance of both accumulation and avoidance responses in lab-grown *Arabidopsis*, as well as several wild plant species (Fig. 4). Detached leaves were irradiated for 1 h with 0.1 or 100 µmol·m^{-2}·s^{-1} of blue light. To examine unspecific changes in leaf bidirectional reflectance, *e.g.* due to measuring light or leaf dehydration, we also measured the effects of the red light applied without actinic blue light. In *Arabidopsis*, both the avoidance and accumulation responses were easily detected, with mean sensitivities $\Delta R/R_0$ of 1.08 (similar to the previous experiment) and -0.23. When measuring light was applied without the actinic beam, no substantial change in cross-polarized reflectance was observed. The ratio $\Delta R/R_0$ and the analogous sensitivity measured for hemispherical transmittance, $\Delta T/T_0$, were comparable in magnitude (Fig. 4 A).

To assess the potential applicability of depolarization-based detection of chloroplast relocations for field studies, we investigated the cross-polarized reflectance changes in detached photosynthetic organs of four wild species, irradiated with 0 (measuring beam only), 0.1 or 100 µmol·m^{-2}·s^{-1} of blue light for 1 h. Measurements were recorded on rosette leaves of *Draba verna* (Fig. 4 B), a close relative of *Arabidopsis*, stem leaves of *Stellaria media* (Fig. 4 C), scales covering flowering shoots of *Tussilago farfara* (Fig. 4 D), and green stems of *Vaccinium myrtillus* (Fig. 4 E). The red measuring beam did not induce substantial changes in reflectance or transmittance in any of the investigated species. Pronounced chloroplast accumulation and avoidance responses were observed through microscopic observations in

D. verna (Fig. 4 B) and *S. media* (Fig. 4 C) leaves. As in *Arabidopsis*, the accumulation response led to a substantial decrease in cross-polarized reflectance and transmittance in both species, while the avoidance response had the opposite effect. In the scales of *T. farfara*, only the avoidance response could be observed microscopically. It was accompanied by an easily detectable increase in reflectance (mean $\Delta R/R_0$ of 0.22) and transmittance (Fig. 4 D). We also investigated chloroplast relocations in green, photosynthetic stems of *Vaccinium myrtillus*, collected in early spring, before bud break. While the transmittance-based method could not be used for opaque stems, the sizeable (mean $\Delta R/R_0$ of 0.3) changes in cross-polarized reflectance induced by irradiation with high blue light pointed to the chloroplast avoidance response. Low blue light led to a decrease in reflectance (mean $\Delta R/R_0$ of 0.06), however, the change in reflectance was not statistically significant from one recorded for mock-irradiated samples. These results suggest that cross-polarized reflectance may be a convenient method for the investigation of chloroplast avoidance in photosynthetic stem tissues. Green stems of *V. myrtillus* can carry out photosynthesis even in winter and early spring when the shoots are leafless and covered with snow (Havas 1971). They are equipped with stomata and photosynthetic tissue located directly beneath the epidermis, a feature shared with several genera of shrubs, inhabiting extreme environments (Nilsen et al. 1995, Ávila et al. 2014). Studies in Norwegian tundra indicate that stem photosynthesis corresponds to ca. 10 – 15% of leaf photosynthesis in summer (Skre 1975). In deciduous shrubs, such as *V. myrtillus*, stem photosynthesis may play an important role in the fall and early spring, when temperatures are low. In such conditions, the sensitivity of chloroplast movements to light in leaves increases (Łabuz et al. 2015).

The effect of chloroplast movements on directional leaf reflectance appears to be substantial, however, the estimation of the magnitude of response requires care. Changes in the signal due to light-induced leaf reorientation may be then mistaken for chloroplast relocations. In this work, we show that measurements of cross-polarized reflectance alleviates this problem, enabling sensitive non-contact detection of chloroplast movements. The developed methodology may be combined with already available hardware for remote sensing to improve the assessment of physiological traits in the field.

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Author contributions

P.H. designed the study. P.H., A.G., and J.L. conducted the research. P.H. and J.L. wrote the manuscript. All authors commented on the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

The data that support the findings of this study are openly available in FigShare at https://doi.org/10.6084/m9.figshare.21082654 (reflectance and transmittance recordings for *Arabidopsis* WT and mutants) and https://doi.org/10.6084/m9.figshare.24424843 (wild plants). Java source code for the software is publicly available via GitHub at https://github.com/pawelHerm/beamJ/tree/master/BeamJ. Wolfram Mathematica package for ray tracing is available at https://github.com/plantPhotobiologyLab/openRayTracer.

References

Banaś, A. K., Aggarwal, C., Łabuz, J., Sztatelman, O., Gabryś, H. (2012). Blue light signalling in chloroplast movements. *Journal of Experimental Botany*, *63*(4), 1559–1574.

Baránková, B., Lazár, D., Nauš, J. (2016). Analysis of the effect of chloroplast arrangement on optical properties of green tobacco leaves. *Remote Sensing of Environment*, *174*, 181–196.

Björkman, O., Powles S. B.. (1981). Leaf movement in the shade species Oxalis oregana. I. Response to light level and light quality. *Carnegie Institution of Washington Year Book 80*, pp. 59 – 62.

Bousquet, L., Lachérade, S., Jacquemoud, S., Moya, I. (2005). Leaf BRDF measurements and model for specular and diffuse components differentiation. *Remote Sensing of Environment*, 98, 201-211.

Brakke T. W. (1994) Specular and diffuse components of radiation scattered by leaves. *Agricultural and Forest Meteorology*, 71, 283-295.

Burbidge, J. B., Magee, L., Leslie R. A. (1988) Alternative transformations to handle extreme values of the dependent variable. *Journal of the American Statistical Association* 83: 123-127.

Carter G. (1991). Primary and Secondary Effects of Water Content on the Spectral Reflectance of Leaves. *American Journal of Botany* 78, 916-924.

Davis, P. A., Caylor, S., Whippo, C. W., Hangarter, R. P. (2011). Changes in leaf optical properties associated with light-dependent chloroplast movements. *Plant, Cell and Environment*, *34*(12), 2047–2059.

Dutta, S., Cruz, J. A., Jiao, Y., Chen, J., Kramer, D., Osteryoung, K. W. (2015) Non-invasive, whole-plant imaging of chloroplast movement and chlorophyll fluorescence reveals photosynthetic phenotypes independent of chloroplast photorelocation defects in chloroplast division mutants. *Plant Journal* 84, 428-442.

Dutta, S., Cruz, J. A., Imran, S. M., Chen, J., Kramer, D. M., Osteryoung, K. W. (2017). Variations in chloroplast movement and chlorophyll fluorescence among chloroplast division mutants under light stress. *Journal of Experimental Botany*, 68(13), 3541-3555.

Gabryś, H., Banaś, A. K., Hermanowicz, P., Krzeszowiec, W., Leśniewski, S., Łabuz, J., Sztatelman, O. (2017). Photometric Assays for Chloroplast Movement Responses to Blue Light. *Bio-Protocol*, *7*(11), 1–11.

Grant, L., Daughtry, C. S.T, Vanderbilt, V. C. (1987). Polarized and non-polarized leaf reflectances of *Coleus blumei*. *Environmental and Experimental Botany* 27: 139-145.

Gorton, H. L., Williams, W. E., Vogelmann, T. C. (1999). Chloroplast movement in *Alocasia macrorrhiza*. *Physiologia Plantarum*, 106, 421–428.

Hart, J. E., Sullivan, S., Hermanowicz, P., Petersen, J., Diaz-Ramos, L. A., Hoey, D. J., Łabuz, J., Christie, J. M. (2019). Engineering the phototropin photocycle improves photoreceptor performance and plant biomass production. *Proceedings of the National Academy of Sciences*, 116(25), 12550-12557.

Havas P. (1971). The water economy of the bilberry (*Vaccinium myrtillus*) under winter conditions. *Annales Universitatis Turkuensis. Series A II, Biologica-geographica-geologica* 47, 41-52.

Hermanowicz, P., Banaś, A. K., Sztatelman, O., Gabryś, H., & Łabuz, J. (2019). UV-B Induces Chloroplast Movements in a Phototropin-Dependent Manner. *Frontiers in Plant Science*, *10*, 1279.

Hothorn T., Bretz F., Westfall P. (2008). Simultaneous Inference in General Parametric Models. *Biometrical Journal* 50, 346-363.

Inoue, Y., Shibata, K. (1973) Light-induced Chloroplast Rearrangements and Their Action Spectra as Measured by Absorption Spectrophotometry. *Planta* 114, 341-358.

Inoue, S. I., Kinoshita, T., Takemiya, A., Doi, M., Shimazaki, K. I. (2008). Leaf positioning of Arabidopsis in response to blue light. *Molecular plant*, 1(1), 15-26.

Jacquemoud, S., and Ustin, S. (2019). Leaf optical properties. Cambridge University Press.

Jarillo, J. A., Gabrys, H., Capel, J., Alonso, J. M., Ecker, J. R., & Cashmore, A. R. (2001). Phototropin-related NPL1 controls chloroplast relocation induced by blue light. *Nature*, 410(6831), 952–954.

Kagawa, T., Sakai, T., Suetsugu, N., Oikawa, K., Ishiguro, S., Kato, T., Tabata, S., Okada, K., Wada, M. (2001). Arabidopsis NPL1: A Phototropin Homolog Controlling the Chloroplast High-Light Avoidance Response. *Science*, *291*(5511), 2138–2141.

Kalshoven, J. E., Tierney, M. R., Daughtry, C. S. T., McMurtrey, J. E. Remote sensing of crop parameters with a polarized, frequency-doubled Nd:YAG laser. *Applied Optics* 34, 2745 -

Lehmann, P., Nöthen, J., Schmidt Von Braun, S., Bohnsack, M. T., Mirus, O., & Schleiff, E. (2011). Transitions of gene expression induced by short-term blue light. *Plant Biology*, *13*(2), 349–361.

Lenth R. V. (2021). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. https://CRAN.R-project.org/package=emmeans

Li, Y., Chen, Y., Huang, J., (2018). An approach to improve leaf pigment content retrieval by removing specular reflectance through polarization measurements. *IEEE Transactions on Geoscience and Remote Sensing*, 57, 2173–2186.

Luda, M. A., Drechsler, M., Schmiegelow, C. T., & Codnia, J. (2019). Compact embedded device for lock-in measurements and experiment active control. *Review of Scientific Instruments*, 90, 023106.

Łabuz, J., Hermanowicz, P., Gabryś, H. (2015). The impact of temperature on blue light induced chloroplast movements in Arabidopsis thaliana. *Plant Science*, 239, 238-249.

Łabuz, J., Sztatelman, O., Hermanowicz, P. (2022). Molecular insights into the phototropin control of chloroplast movements. *Journal of Experimental Botany*, 73(18), 6034-6051.

Neuwirthová E, Lhotáková Z, Lukeš P, Albrechtová J. (2021). Leaf Surface Reflectance Does Not Affect Biophysical Traits Modelling from VIS-NIR Spectra in Plants with Sparsely Distributed Trichomes. *Remote Sensing*, 13(20), 4144.

Newman J. P., Fong M., Millard D. C., Whitmire C. J., Stanley G. B., Potter S. M. (2015) Optogenetic feedback control of neural activity. *eLife* 4, e07192.

Nicodemus, F. E. (1965). Directional reflectance and emissivity of an opaque surface. *Applied Optics*, 4(7), 767-775.

Nilsen, E. T. (1995). Stem photosynthesis: extent, patterns, and role in plant carbon economy. In Plant Stems (pp. 223-240). Academic Press.

Park, Y., Chow, W. S., Anderson, J. M. (1996). Chloroplast Movement in the Shade Plant Tradescantia albiflora Helps Protect Photosystem II against Light Stress. *Plant Physiology*, *111*, 867–875.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team (2019). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-143, https://CRAN.R-project.org/package=nlme.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rowell, R. L, Levit, A. B., Aval, G. M. (1970) Determination of the Absolute Alignment of a Polarizer. *Applied Optics*, 9, 1708.

Rvachev, V. P., Guminetskii, S. G. (1966). The structure of light beams reflected by plant leaves. *Journal of Applied Spectroscopy* 4(5), 303-307.

Sakai, T., Kagawa, T., Kasahara, M., Swartz, T. E., Christie, J. M., Briggs, W. R., Wada, M., & Okada, K. (2001). Arabidopsis nph1 and npl1: Blue light receptors that mediate both phototropism and chloroplast relocation. *Proceedings of the National Academy of Sciences*, 98(12), 6969–6974.

Schaepman-Strub, G., Schaepman, M. E., Painter, T. H., Dangel, S., & Martonchik, J. V. (2006). Reflectance quantities in optical remote sensing—Definitions and case studies. *Remote sensing of environment*, 103(1), 27-42.

Seybold, A. (1955). Beiträge Hat die Chloroplastenverlagerung in Laubblättern eine Bedeutung? *Naturwissenschaften* 43(4), 90-91.

Skre, O. (1975) CO_2 exchange in Norwegian tundra plants studied by infrared gas analyzer technique. *Fennoscandian Tundra Ecosystems: Part 1 Plants and Microorganisms.* Berlin, Heidelberg: Springer Berlin Heidelberg, 168-183.

Шульгин И.А., Молдау Х.А. (1965) К вопросу о спектральных коэффициентах яркости листьев растений в естественном и поляризованном свете. *Доклады Академии наук* 162, 1430 – 1433.

Stewart W.S., Banister P. (1973). Seasonal Changes in Carbohydrate Content of Three Vaccinium spp with Particular Reference to V. uliginosum L. and its Distribution in the British Isles, Flora, 162, 134-155.

Suetsugu, N., Kagawa, T., Wada, M. (2005). An Auxilin-Like J-Domain Protein, JAC1, Regulates Phototropin-Mediated Chloroplast Movement. *Plant Physiology*, 139, 151–162.

Vanderbilt, V. C., Grant, L., Daughtry, C. S. T. (1985). Polarization of light scattered by vegetation. *Proceedings of the IEEE*, 73(6), 1012-1024.

Walczak T., Gabryś H. (1980). New type of photometer for measurement of transmission changes corresponding to chloroplast movements in leaves. *Photosynthetica* 14, 65□72.

Williams, W. E., Gorton, H. L., Witiak, S. M. (2003) Chloroplast movements in the field. *Plant, Cell and Environment* 26, 2005-2014.

Wu, T., Zhang, L., Cen, Y., Huang, Ch., Sun, X., Zhao, H., Tong, Q. (2013). Polarized spectral measurement and analysis of *Sedum spectabile* Boreau using a field imaging spectrometer system. *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing* 6, 724-730.

Zurzycki J (1961) The influence of chloroplast displacements on the optical properties of leaves. *Acta Societatis Botanicorum Poloniae* 30, 503-527.

Figures, including figure legends

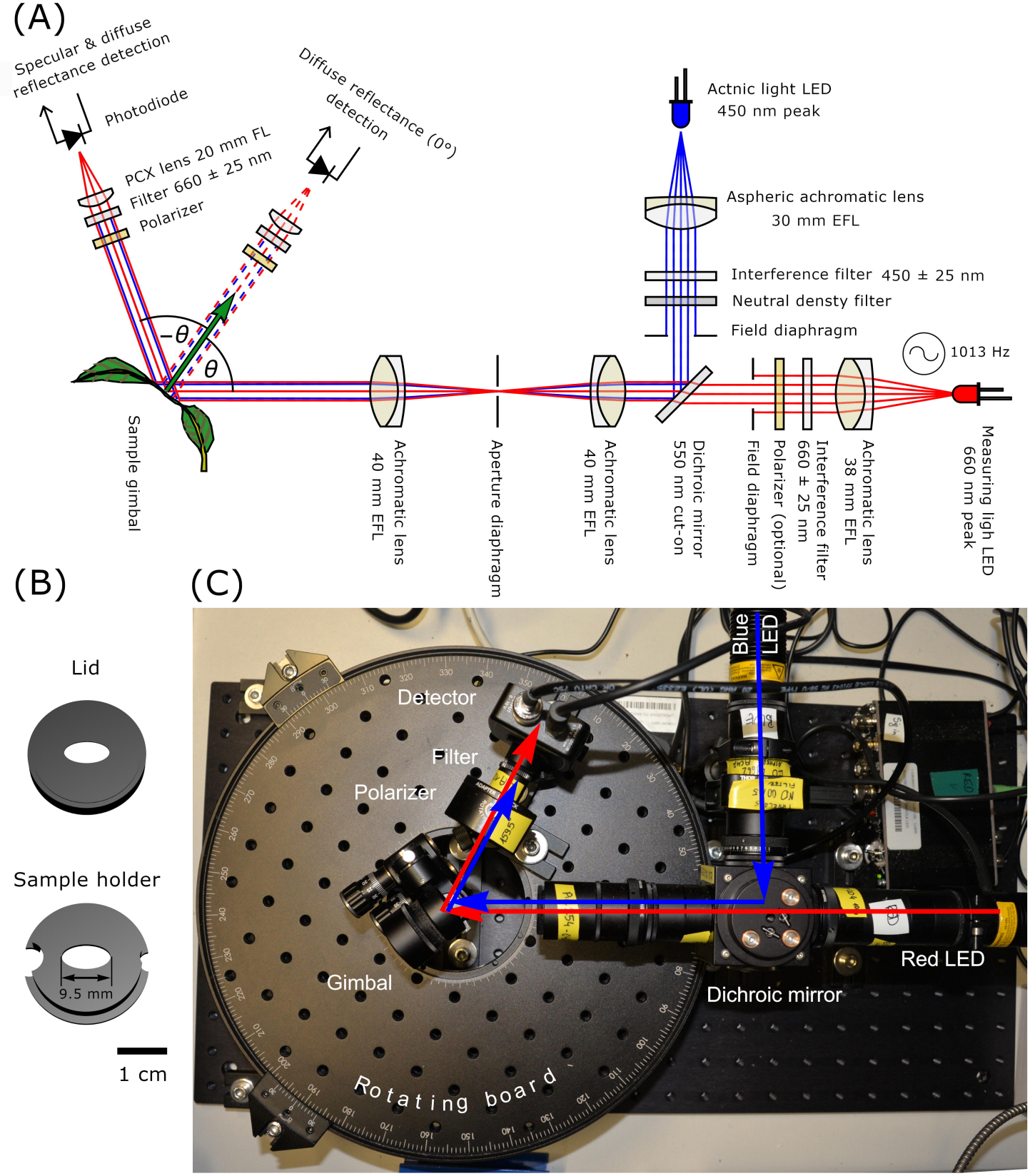
Fig. 1. (A) Schematic representation of the reflectometric set-up used in the study. Blue and red beams are collimated, combined using a dichroic mirror, and directed towards the leaf. Red light, modulated at 1013 Hz, is used to monitor reflectance changes due to chloroplast movements. Blue, continuous

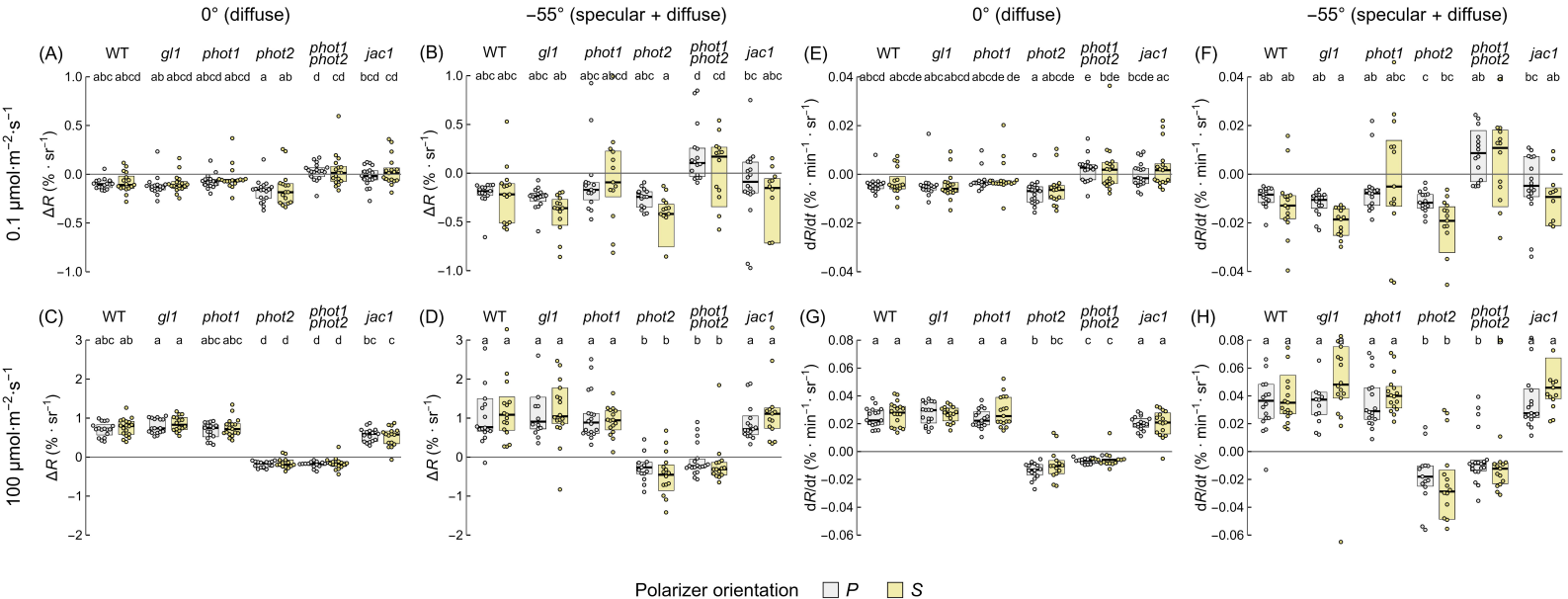
light induces chloroplast movements. The angle of incidence θ of the combined blue and red beams onto the surface of the leaf is either 40° or 55°. The directional reflectance is measured at a variable angle of observation, with the detector lying in the plane spanned by the direction of incident rays and the normal to the leaf. Reflected light passes through a linear polarizer, an interference filter (blocking blue actinic light and attenuating chlorophyll fluorescence), and a plano-convex lens, which focuses reflected light on the detector. (B) A model of the 3D printed sample holder used to mount the leaf in the gimbal. The holder features a hole to prevent reflection from the material beneath the leaf. (C) Photo of the set-up. The rotating board provides control over the polar angle at which reflected light is detected.

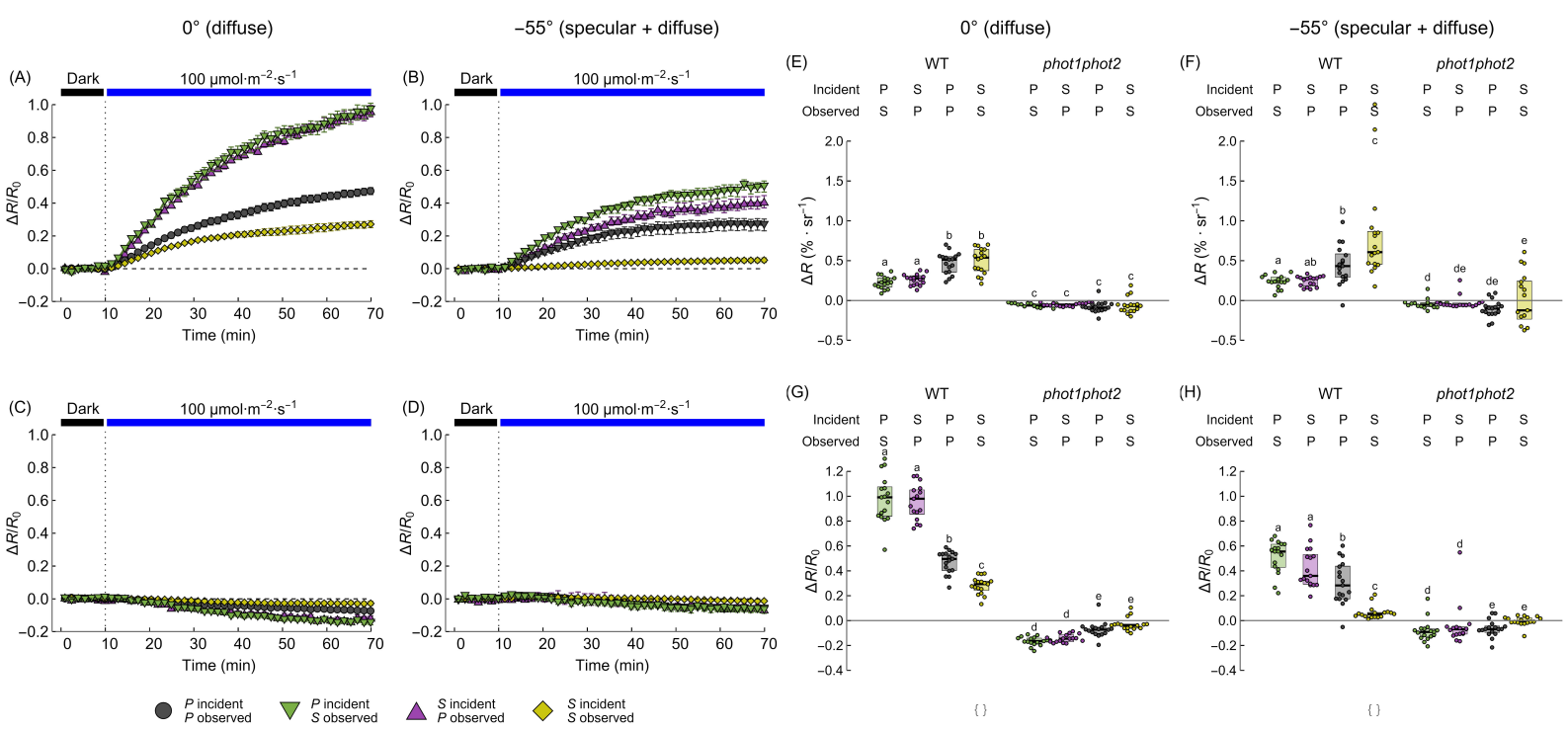
Fig. 2. The amplitudes (A - D) and maximal rates (E - H) of red-light bidirectional reflectance changes induced by 1 h irradiation with blue light of 0.1 (A, B, E, F) or 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (C, D, G, H) in rosette leaves of dark-adapted *Arabidopsis thaliana* plants: wild-type (WT), *glabra1* and chloroplast movement mutants (*phot1*, *phot2*, *phot1phot2*, *jac1*). The blue actinic and red measuring beams impinged the adaxial leaf surface at the angle of 55°. For measurements of diffuse directional reflectance (A, C, E, G), the detector was positioned at 0° to the leaf's normal. For measurements of combined specular and diffuse directional reflectance (B, D, F, H), the detector was positioned at -55°. For each detector position, two polarizer orientations were used: the transmission axis of the polarizer was either parallel to the plane of incidence (*P*, attenuates specular reflection, grey boxes) or perpendicular (*S*, yellow boxes). Each dot represents a value obtained for one leaf, with a single leaf harvested from each plant. Boxes show interquartile ranges, while the horizontal bars mark medians. Groups that do not share any letter represent groups for which the means of transformed values differ at the 0.05 level (Tukey's method, adjusted for multiple comparisons).

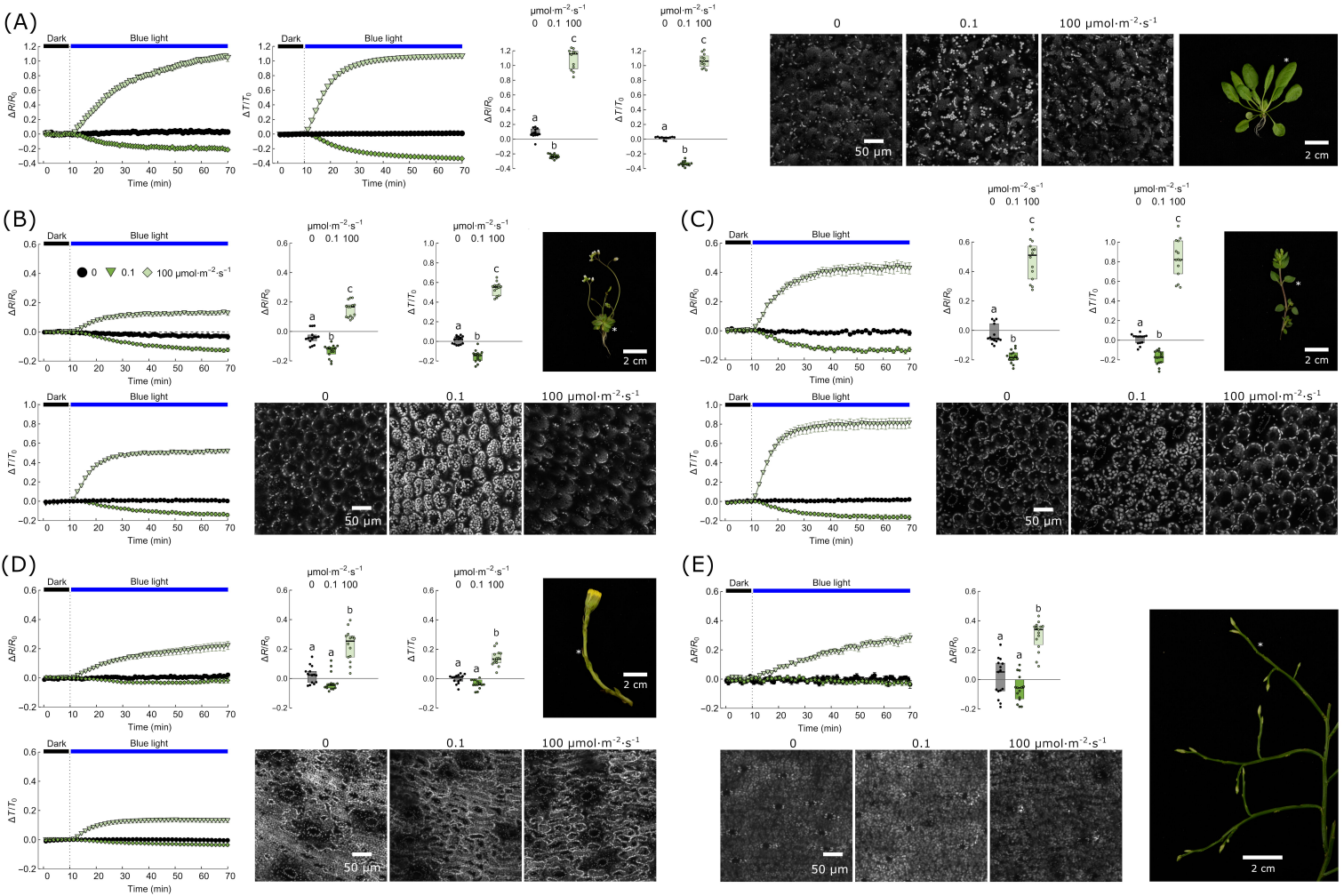
Fig. 3. Changes in the polarized red-light bidirectional reflectance induced by 1 h irradiation with high blue light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of the adaxial surface of rosette leaves of 5-week-old wild-type (WT) (A, B) and *phot1phot2* (C, D) *Arabidopsis* plants. The blue actinic and red measuring beams impinged the adaxial leaf surface at the angle of 55°. For measurements of diffuse reflectance (A, C, E, G), the detector was positioned at 0° to the leaf normal. For measurements of combined specular and diffuse reflectance (B, D, F, H), the detector was positioned at -55°. Four possible combinations of the orientation (*P/S*) of the polarizers in front of the measuring light source and the detector were examined. Reflectance change curves, shown for WT (A, B) and *phot1phot2* (C, D), were normalized by the initial reflectance R_0 of dark-adapted leaves. (E, F) Raw ΔR and (G, H) normalized amplitudes $\Delta R/R_0$ of reflectance changes. Each dot represents a value obtained for one leaf, with a single leaf harvested from each plant. Boxes show interquartile ranges, while the horizontal bars mark medians. Boxes that do not share any letter represent groups for which the means of transformed values differ at the 0.05 level (Tukey's method, adjusted for multiple comparisons).

Fig. 4. Chloroplast movements examined with photometric, reflectometric, and microscopic observations, in laboratory-grown *Arabidopsis thaliana* (A), and plants collected in the field: *Draba verna* (B), *Stellaria media* (C), *Tussilago farfara* (D), and *Vaccinium myrtillus* (E). For each species: Time courses of changes in red-light bidirectional reflectance and hemispherical transmittance induced by 1 h irradiation with low (0.1) or high blue light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or mock irradiation of the sun-exposed side of leaves (adaxial – *Arabidopsis*, *Draba*, *Stellaria*; abaxial - *Tussilago*) or stems (*Vaccinium*). The angle of incidence of beams was 55° while the angle of observation was 0° (diffuse reflectance recorded). The transmission axis of the polarizer was parallel to the plane of incidence (*P*) for the light source and perpendicular to the detector (*S*). Each dot represents a value obtained for one leaf harvested from a different plant. Boxes show interquartile ranges, while the horizontal bars mark medians. The means of transformed values from groups that do not share any letter differ at the 0.05 level (Tukey's method, adjusted for multiple comparisons). Confocal microscopy images of leaf tissue or stems for *Vaccinium* after 1 h irradiation with low blue light (0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or high blue light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or mock irradiated. Photographs show the developmental stage of plants at the time of the measurement, with the examined organs marked with asterisks.


(A)
Specular & diffuse reflectance detection
Photodiode
PCX lens 20 mm FL
Filter 660 ± 25 nm
Polarizer
Diffuse reflectance (0°) detection
−θ
θ
Sample gimbal
Achromatic lens 40 mm EFL
Aperture diaphragm
Achromatic lens 40 mm EFL
Dichroic mirror 550 nm cut-on
Field diaphragm
Polarizer (optional)
Interference filter 660 ± 25 nm
Achromatic lens 38 mm EFL
Measuring ligh LED 660 nm peak
1013 Hz
Field diaphragm
Neutral densty filter
Interference filter 450 ± 25 nm
Aspheric achromatic lens 30 mm EFL
Actnic light LED 450 nm peak
(B)
Lid
Sample holder
9.5 mm
1 cm
(C)
Detector
Filter
Polarizer
Gimbal
Rotating board
Dichroic mirror
Red LED
Blue LED


0° (diffuse)
−55° (specular + diffuse)
0° (diffuse)
−55° (specular + diffuse)
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
WT
gl1
phot1
phot2
phot1 phot2
jac1
0.1 μmol·m⁻²·s⁻¹
100 μmol·m⁻²·s⁻¹
ΔR (% · sr⁻¹)
dR/dt (% · min⁻¹ · sr⁻¹)
Polarizer orientation
P
S


0° (diffuse)
−55° (specular + diffuse)
0° (diffuse)
−55° (specular + diffuse)
(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
Dark
100 µmol·m⁻²·s⁻¹
ΔR/R0
Time (min)
WT
phot1phot2
Incident
Observed
ΔR (% · sr⁻¹)
P incident P observed
P incident S observed
S incident P observed
S incident S observed


(A)
Dark
Blue light
ΔR/R0
ΔT/T0
Time (min)
μmol·m-2·s-1
0 0.1 100
a
b
c
0
0.1
100 μmol·m-2·s-1
50 μm
2 cm
(B)
0 0.1 100 μmol·m-2·s-1
(C)
(D)
(E)