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Dihydroisocoumarin Content and Phenotyping of *Hydrangea macrophylla* subsp. *serrata* Cultivars under Different Shading Regimes

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Abstract: Hortensias (*Hydrangea macrophylla* L.) are well known as ornamental plants with their impressive flowers. Besides being an ornamental plant, some hortensia species contain constituents of nutritional and pharmaceutical interest. In this context, *H. macrophylla* subsp. *serrata* contains dihydroisocoumarins (DHCs), in particular hydrangenol (HG) and phyllodulcin (PD), which determine produce quality. For the successful cultivation of *H. macrophylla* subsp. *serrata*, shading may be required. The response of *H. macrophylla* subsp. *serrata* as a source for DHCs was investigated in two growing seasons using three different cultivars ('Amagi Amacha', 'Oamacha' and 'Odoriko Amacha') under three different light conditions: no shade (100% photosynthetic active radiation, PAR), partial (72% PAR) and full shading (36% PAR). The shading regimes had no significant effect on dihydroisocoumarin content in leaf dry matter in each single cultivar. However, 'Amagi Amacha' and 'Oamacha' yielded significantly higher PD content in comparison to 'Odoriko Amacha', which showed, in contrast, the significantly highest HG content. The total biomass was not significantly affected by the shading regime, but slightly higher biomass was observed under partially shaded and full-shade conditions. Hyperspectral vegetation indices (VIs) and color measurements indicate less vital plants under no shade conditions. While lighting is an important growth factor for hortensia production, DHC is cultivar dependent.

Keywords: *Hydrangea macrophylla* subsp. *serrata*; phenotyping; vegetation indices; color measurement; light conditions; dihydroisocoumarins; hydrangenol; phyllodulcin

1. Introduction

Hydrangea macrophylla subsp. *serrata* originates in Japan, where it is used as Amacha (which is Japanese with 甘い = amai = sweet, 茶 = cha = tea), a sweet-tasting tea in rituals surrounding ceremonies on the birthday of the Buddha, Hanamatsuri in Japanese [1]. The originally wildly growing plant is nowadays cultivated in smaller gardens [2]. Various common names have been proposed for *H. macrophylla* subsp. *serrata*, such as San-soogook, Mountain Hydrangea, Tea of Heaven [3] and Amacha [4], to make it more distinguishable from other species of the same genus.

To distinguish the phyllodulcin (PD)-containing *H. macrophylla* subsp. *serrata* from other plants from the genus *Hydrangea*, different parameters are to be considered. First, a taxonomic and genetic clarification is needed. The nomenclature of *Hydrangea* was revised by McClintok, as the genus *Hydrangea* can be found around the world, from eastern Asia to northern America as well as tropical regions on the northern and southern hemispheres. Adding to this spreading of *Hydrangea* in connection to a multitude of different cultivars has led to a confusion in the nomenclature [5]. This revision concluded 23 species in the genus of *Hydrangea*. One of these is *H. macrophylla* subsp. *serrata*, also known as *H. serrata*. Additional revisions and reviews tried to clarify the status of the genus of the hortensias (e.g., [6–8]). Whilst the nomenclature is not clearly defined, *H. macrophylla* subsp. *serrata* seems to be the appropriate nomenclature in scientific publications. Second, on a morphological level, a separation of *H. serrata* and *H. macrophylla* might be justified. *H. macrophylla* subsp. *serrata* seems to be the scientifically substantiated nomenclature from a genetic point of view [9]. Still, *H. serrata* is used frequently in scientific writings (e.g., [10–15]) and for convenience [6,9].

Third, besides the taxonomic clarification, the possible usage for Amacha tea is the main criterion for a *Hydrangea* to qualify as a *H. macrophylla* subsp. *serrata*. The characteristic taste of the Amacha is mainly formed by phyllodulcin (PD), a dihydroisocoumarin (DHC) derivate [1]. PD yield varies within a cultivar over the year, with the highest reported amounts in July, followed by August and June. Additionally, different parts of the plant contain different amounts of PD, as sepals of display flowers and buds contain significantly more PD than young and old leaves [2]. Dihydroisocoumarins are biosynthetically synthesized, starting from the shikimic acid pathway via coumaric acid as well as from mevalonate with a specific PKS-type enzyme with stilbenecarboxylates as intermediates, as reported by Kindl [16]. Besides PD, hydrangenol (HG), a precursor of PD [17] that is also present in other *Hydrangea* species (e.g., *H. macrophylla* 'Engel's White') [18], is of interest in this context. In the context of the present study, the occurrence of PD is the main criterion for a *Hydrangea* to be eligible for grouping as tea-hortensia.

The lighting regime, and especially UV lighting, is an important factor for improving produce quality in horticultural production [19]. Abiotic stress caused by high light intensity can be managed through shading [20]. In commercial *Hydrangea* production, shading is essential to prevent wilting, but higher levels of shading will decrease flowering [21] or could lead to flower greening [22]. It has been found that in the medicinal plant guaco (*Mikania glomerata*), coumarin content could be increased by reduced UV-A and UV-B radiation [23]. Moreover, short wavelength radiation (UV-C and UV-B) was shown to have an increasing effect on isocoumarins in fresh-cut carrots, which was higher when combined with wounding [24]. This combinatorial effect of PAR and UV in the lighting regime on plant growth and DHC synthesis in *H. macrophylla* subsp. *serrata* has to be considered, because light quality as a cultivation factor can be used to control product quality and yield [25]. Therefore, it seems possible that light exposure may in part have an influence on DHC content and, hence, produce quality. Shading reduces the photosynthetically active radiation (PAR, 400–700 nm). This reduction is the main criterion to differentiate shading materials [26]. The reduction of UV radiation limits plant stress and promotes healthy and vital plants [27]. Shading is used to decrease evaporation, control plant growth and manipulate the microclimate by reducing day temperature, increasing night temperature and affecting water vapor and CO_2 concentration [28]. During the production of ornamental plants, the influence of light scattering, mainly for shaping and flowering control, is used [29].

Plant phenotyping provides a tool for health assessment in plants, for example, by monitoring leaf traits [30], photosynthesis [31] or stress [32]. Vegetation indices provide information about a multitude of different plant parameters. These indices are calculated using different mathematical operations on reflectance spectra [33]. One of the commonly used vegetation indices is the Normalized Difference Vegetation Index (NDVI). The interpretation of the NDVI allows for assessments of plant health and productivity by assessing chlorophyll and nitrogen content and PAR absorption, as well as potential photosynthetic activity [34]. The Plant Senescence Reflectance Index (PSRI) is calculated to illustrate plant senescence during a vegetation period and is most sensitive toward the end of plant development [35]. The Photochemical Reflectance Index (PRI) is mainly used as a proxy for light-use efficiency [36]. Leaf pigment-related indices can be grouped according to Roberts et al. to distinguish chlorophyll, carotenoids and anthocyanin [37]. The Red Edge Inflection Point is mainly dependent on chlorophyll but is also responsive to additional biological features, such as plant species and cultivar, plant age and leaf N content [38,39]. Pigment changes can also be measured by leaf color [40], which can be described by hue angle (h°). Hue angle illustrates a color gradient from 0 or 360° hue (red) to 180° hue (green) [41].

We hypothesized that *H. macrophylla* subsp. *serrata* may require at least some shading to optimize plant performance and biomass production to gain higher PD content and higher PD yield per area. In this context, the main focus lies on DHC yield as a function of DHC content in the leaves and leafy biomass. Moreover, the lighting regime directly affects plant performance and biomass yield and that the three different cultivars ('Amagi Amacha', 'Oamacha' and 'Odoriko Amacha') react differently to the increased sun exposure. To evaluate the response of *H. macrophylla* subsp. *serrata* to different light exposures, non-invasive plant phenotyping was carried out.

In this study, we compare for the first time three different *H. macrophylla* subsp. *serrata* cultivars, their response to three different lighting regimes on biomass production and DHC content. Non-invasive sensor measurements were used to characterize the lighting effects in more detail.

2. Materials and Methods

2.1. Plant Material/Cultivation

Terminal cuttings of *Hydrangea macrophylla* subsp. *serrata* cultivars 'Amagi Amacha', 'Oamacha' and 'Odoriko Amacha' were obtained in spring from Kötterheinrich-Hortensienkulturen e.K. (Lengererich, Germany). The cuttings were planted in 3 l pots in "Einheitserde ED73" substrate (Einheitserdewerke Werkverband e.V., Sinntal-Altgronau, Germany) and cultivated in a greenhouse until the start of the experiment. For both years, 2018 and 2019, the experiments started with new cuttings that were trimmed to a homogenous height of approximately 15 cm before the experiment. Measurements started on day of year (DOY) 228 in both years. Different shading regimes were implemented on DOY 232 in 2018 and DOY 228 in 2019. From these DOYs, on dates are referred to as DALE (days after light exposure). Accordingly, DOY 233 in 2018 equals 1 DALE. In 2018, the experiment ended on 264 DOY (32 DALE) and in 2019 on DOY 260 (32 DALE). Conversions of DOY, DALE and dates can be found in the Supplementary Materials. Figure 1 illustrates hours of sunshine over the course of the experiment in 2018 (mean hours of sunshine: 9.48) and 2019 (mean hours of sunshine: 9.37). Figure 2 displays mean daily temperatures in 2018 and 2019. Except for daily variations, both years showed similar development in hours of sunshine and temperatures.

2.2. Experimental Setup

The experiments were conducted in August and September 2018 and 2019 at Campus Klein-Altendorf, University of Bonn, Germany. Plants were exposed to three different levels of light intensity in field experiments. One-third of the plants were exposed to natural sun light (no shade). Partial shade for plants was achieved by using the Haygrove tunnel. The last third of hortensias were cultivated in a Haygrove tunnel with an added black shading net (full shade). A full scheme of the experimental setup is displayed in Figure 3.

Shading levels were quantified with a Gigahertz-Optik $X1_2$ Optometer (Gigahertz-Optic; Member of the GERGHOF GROUP, Türkenfeld, Germany). UV-A and UV-B were measured in W m^{-2} and PAR in photosynthetic photon flux density (PPFD) in µmol m^{-2} s^{-1}. For each lighting treatment, a total of 15 plants were cultivated. From these, a total of five plants were randomly selected by chance before the start of the experiment and used for measurements. Measurements were taken between 8:00 a.m. and 12:00 p.m.

CET with the same sequence of measurements to prevent the effects of daytime. A Sartorius LE1003S scale (Sartorius AG, Göttingen, Germany) was used to perform biomass quantification after cutting plants on ground level.

2.3. Plant Phenotyping

For hyperspectral measurements, the PolyPen RP 400 (UV–VIS) by Photon Systems Instruments (Brno, Czech Republic) was used. This non-imaging spectroreflectometer displays reflectance in a range of 380–780 nm. From the achieved spectra, different vegetation indices as proxies for plant status and stress were calculated. In this study, the last day of measurements was at 32 DALE, as the plants response to different lighting regimes was expected to be the most prominent at this point. Vegetation indices over the course of the trial can be found in the Supplementary Materials. The vegetation indices used in this study can be found in Table 1.

In addition to the PolyPen RP 400 (UV–VIS), color measurements, presented by hue°, were performed using an X-Rite i1 pro (Grand Rapids, MI, USA). Hue° was calculated based on McGuire [41] using MS Excel 2019.

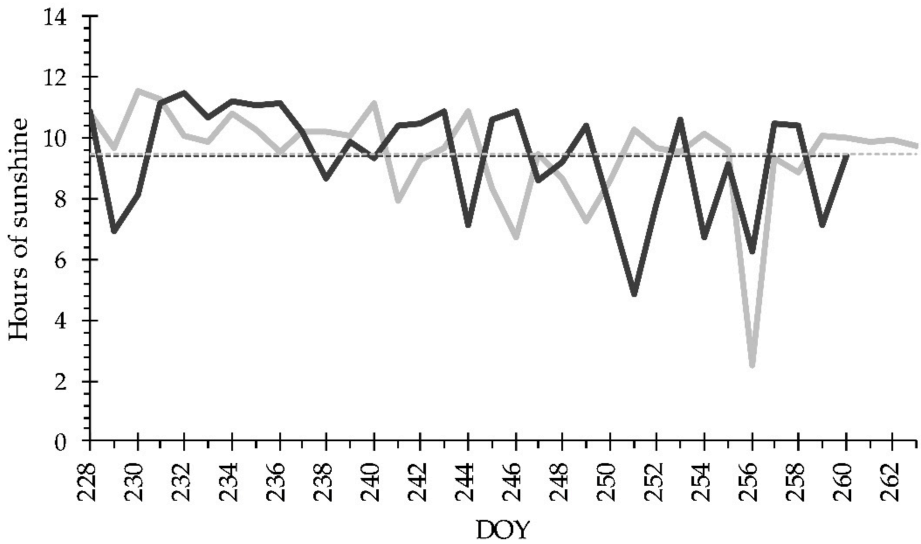


Figure 1. Hours of sunshine (hour of sunshine >120 W m^{-2}) in 2018 (light gray) and 2019 (dark gray) over the course of the experiment (in DOY). Dotted lines represent mean hours of sunshine for the years. Complete data are available via the Supplementary Materials (Table S1).

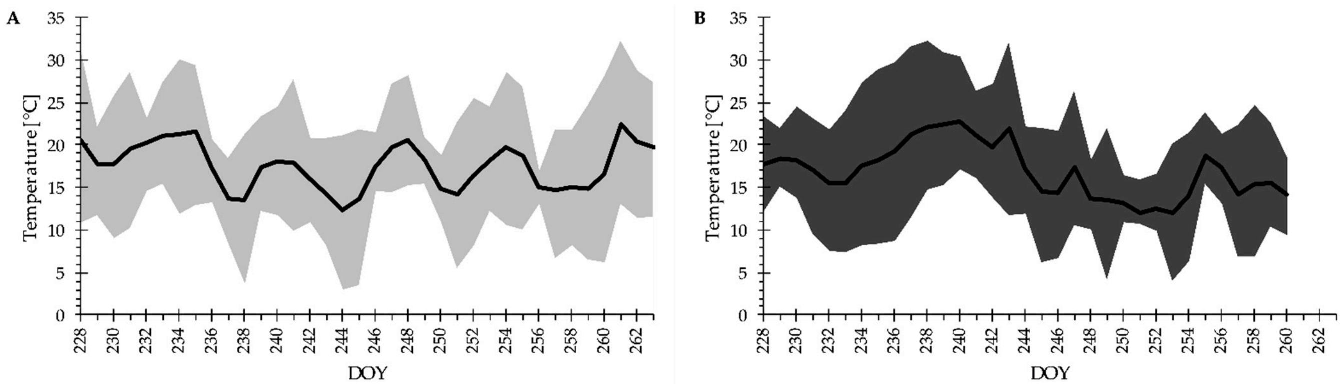
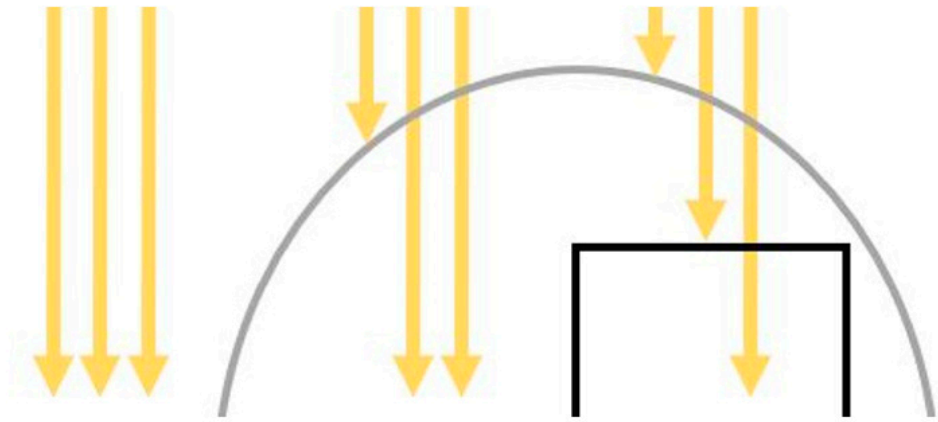


Figure 2. Mean daily temperature (°C) in 2018 (**A**) and 2019 (**B**). Shaded area indicates minimum and maximum temperatures. Complete data are available via the Supplementary Materials (Table S1).



	No Shade		Partial Shade		Full Shade	
UV-A	32.06 ± 8.63	100%	21.33 ± 4.41	67%	11.11 ± 1.85	35%
UV-B	0.75 ± 0.21	100%	0.5 ± 0.08	66%	0.26 ± 0.04	35%
PAR	1433.33 ± 496.1	100%	1026.6 ± 276.09	72%	509.4 ± 115.72	36%

Figure 3. Shading quantification of no shade, partial shade and full shade for UV-A (W m^{-2}), UV-B (W m^{-2}) and PAR (μmol $m^{-2}s^{-1}$) in absolute values including standard deviation and in relative measures to no shade conditions. Irradiation data can be found in the Supplementary Materials (Table S2, irradiation data).

Table 1. Hyperspectral vegetation indices of leaf-based plant phenotyping used in this study, including equations and references.

Category [1]	Vegetation Index	Abbreviation and Equation [2]	Reference
Structure	Normalized Difference Vegetation Index	$NDVI = \frac{(R_{NIR} - R_{RED})}{(R_{NIR} + R_{RED})}$	[42]
Chlorophyll	Modified Chlorophyll Absorption in Reflectance Index 1	$MCARI\,1 = 1.2 * (2.5 * (R_{790} - R_{670}) - 1.3 * (R_{790} - R_{550}))$	[43]
Chlorophyll	Greenness Index	$G = \frac{R_{554}}{R_{677}}$	[44]
Anthocyanins	Anthocyanin Reflectance Index 1	$ARI\,1 = \frac{1}{R_{550}} - \frac{1}{R_{700}}$	[45]
Carotenoids	Carotenoid Reflectance Index 1	$CRI\,1 = \frac{1}{R_{510}} - \frac{1}{R_{550}}$	[46]
Light use	Photochemical Reflectance Index	$PRI = \frac{(R_{531} - R_{570})}{(R_{531} + R_{570})}$	[47]
Stress	Plant Senescence Reflectance Index	$PSRI = \frac{R_{678} - R_{500}}{R_{750}}$	[48]
Stress	Red Edge Inflection Point 1	$REIP\,1 = 700 + 40 * (\frac{\left(\frac{R_{670} + R_{780}}{2}\right) - R_{700}}{R_{740} - R_{700}})$	[49]

[1] Indices are grouped according to Roberts et al. (2011) [37]. [2] In this study: RED = 630, NIR = 780.

2.4. DHC Quantification

For the quantification of DHC content, namely HG and PD, the upper two (fully developed) leaves were harvested by hand on DOY 261 in both years (with an additional late harvest on DOY 281 in 2018) and dried at 40 °C for 72 h. Subsequently, samples of at least 10 mg were homogenized using a mortar and were moistened and fermented before being analyzed. Fermentation was carried out by adding water (200 μL) and finally stopped with methanol (1800 μL), followed by ultrasonic extraction (BANDELIN SONOREX, Berlin, Germany) for 30 min and filtration (membrane filter Chromafil XtraPTFE- 20/25). UPLC analyses of samples were performed on a Waters Acquity UPLC® I-Class System equipped with an Acquity UPLC ελ PDA detector and a commercially available reversed phase C18 column (Luna Omega 1.6 µm Polar C18 50 × 2.1 mm). A binary solvent system consisting of acidified water (0.1% formic acid; A) and acetonitrile (B) was used. The details on the gradient and evaluation program are given in Table 2. The detection wavelength was set at 254 nm, and the chromatographic data were processed by Empore™ 3 Pro 2010.

Table 2. Gradient table of the UPLC evaluation program.

Time	Flow (mL/min)	%A	%B
0.00	0.8	70%	30%
2.00	0.8	66%	34%
2.01	0.8	05%	95%
3.01	0.8	05%	95%
3.02	0.8	70%	30%
4.00	0.8	70%	30%

2.5. Statistical Analysis

The experiments were conducted in a randomized design, with five replications per treatment (n = 5) and each replicate consisting of four independent measurements. In all figures and tables, data are presented as mean and standard deviation ($\overline{x} \pm \sigma$) and the number of replicates (n) are given. One-way analysis of variance (ANOVA) was used to compare means of treatments. Under the given normal distribution (Kolmogorov-Smirnov test) and homoscedasticity (Levene test), a Tukey HSD test or Scheffé test was used as a post hoc procedure to determine homogeneous subgroups at a p-value of $p \leq 0.05$. A t-test was conducted for color measurement at a p-value of $p \leq 0.05$, and significant differences are marked with an asterisk (*). Exact p-values for each experiment are reported in Tables S6 and S7. All statistical analyses were performed using IBM SPSS 26.0 software.

3. Results and Discussion

All results presented are shown with respect to light intensity as influenced by different shading regimes. With a sum of global radiation (Wh/m^2) of about 132,200 in 2018 and 137,500 in 2019 and hours of sunshine of approximately 312 h in 2018 and 309 in 2019, both years revealed similar values from DOY 228 (−4 DALE in 2018 and 0 DALE in 2019) until DOY 260 (28 DALE in 2018 and 32 DALE in 2019). The growth degree analysis showed that 2018 achieved 172.7 h above 12 °C and 2019 reached 163.0 h. From these calculations and the data for hours of sunshine (Figure 1) and temperatures (Figure 2), it can be concluded that the differences between both years do not have a major impact on the outcome of the measurements. Therefore, differences in weather are not be discussed.

3.1. DHC Content

H. macrophylla subsp. *serrata* cultivars used as a feedstock are mainly compared for their PD content. A study by Ujihara et al. (1995) analyzing different leaf ages regarding PD content revealed that younger leaves contain higher percentages of PD in leaf dry matter than older leaves, while flowers and buds had significantly higher content (µg/g FW) than leaves [2]. Because of these reported differences, the effect of leaf age was investigated in 2018 for three cultivars under different lighting regimes. In general, it was confirmed that younger leaves from the upper third of the plant contain more PD than leaves that were taken from the middle or lower third of the plants (Figure 4). With the exception of 'Amagi Amacha', the differences between leaves taken from the middle third and lower third were lower. There was a strong effect of cultivar regarding PD content. 'Amagi Amacha' showed the highest PD content, followed by 'Oamacha' and 'Odoriko Amacha' for all shading treatments and leaf age. The results for leaf age are in accordance with published data [2]. A multivariate testing of the main effects showed only significant interactions for cultivar and leaf position (Table S7), but not for shading and leaf position or for cultivar, shading, and leaf position (Table S7), a detailed analysis of leaf position was only performed in 2018.

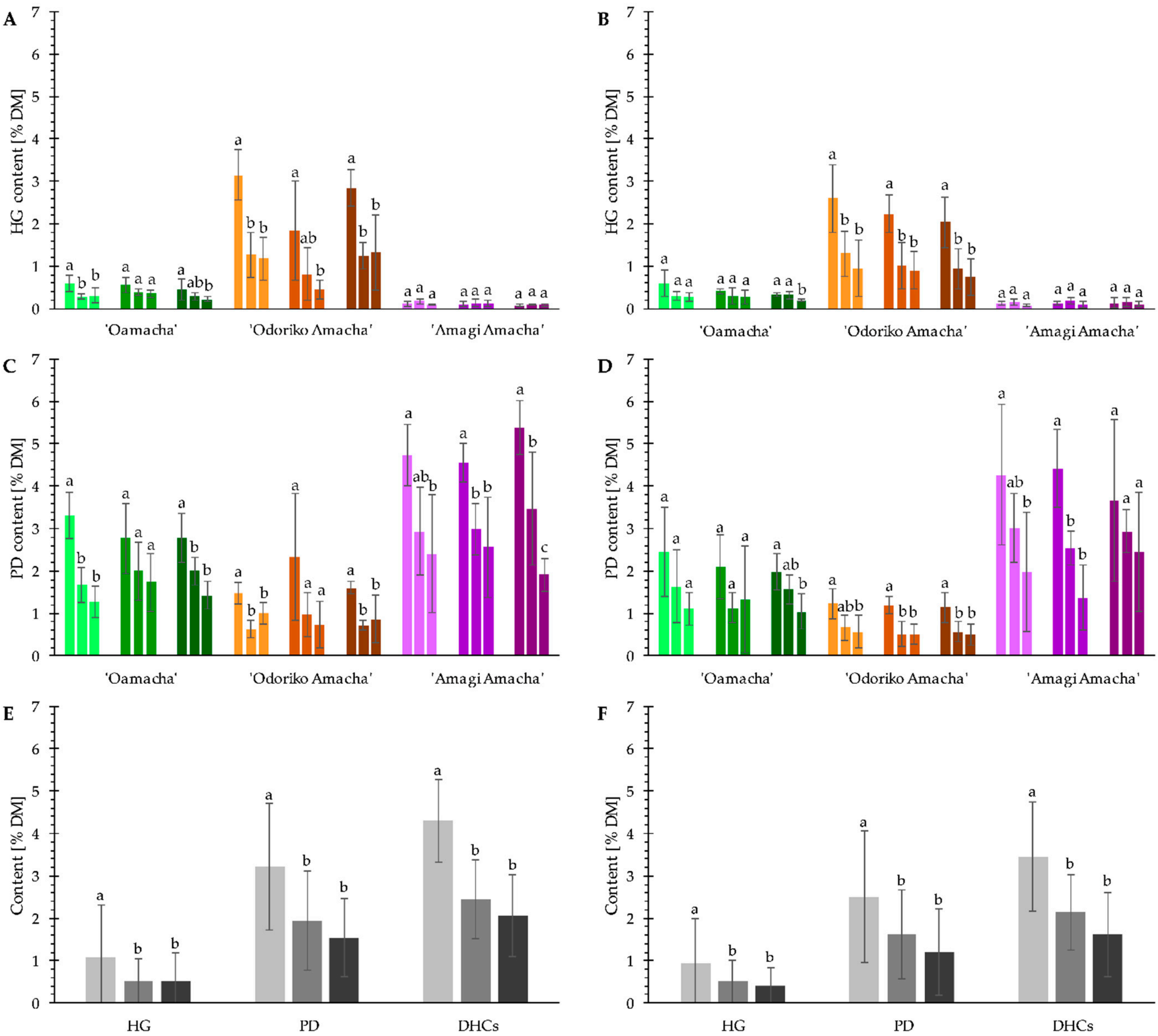


Figure 4. Content (%) of (**A**,**B**) hydrangenol (HG) and (**C**,**D**) phyllodulcin (PD) for three H. macrophylla subsp. serrata cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') and (**E**,**F**) for HG, PD and DHC content pooled over all cultivars depending on three shading scenarios in leaf dry matter for two different harvest dates in 2018 ((**A**,**C**,**E**): 18 September 2018 and (**B**,**D**,**F**): 8 October 2018). (**A**–**D**): Shading scenarios (bright coloring: no shade, light coloring: partial shade, dark coloring: full shade). (**E**,**F**): Differences for leaf position depending on shading pooled over all cultivars (bright gray: no shade, light gray: partial shade, dark gray: full shade). Significant differences for leaf position (left: upper third, middle: middle third, right: lower third) of each cultivar (**A**–**D**) or pooled by cultivar (**E**–**F**) and year calculated by ANOVA and Tukey HSD (n = 5, α = 0.05) are indicated by different letters. Detailed statistical results can be found in the Supplementary Materials for the multivariate analysis (Table S7) and ANOVA tables (Tables S6 and S8).

Besides PD, HG was also assayed for different leaf stages on the plants. HG content was significantly higher in 'Odoriko Amacha' in comparison to that of 'Amagi Amacha' and 'Oamacha. 'Oamacha' and 'Odoriko Amacha' contained higher percentages of HG in young leaves compared to old leaves (Figure 4). 'Amagi Amacha' did not show significantly different contents of HG due to very low HG levels (Figure 4). To some extent, HG exhibits positive protective effects against pathogens such as *Pythium* [50]. Besides that, HG seems to have nearly no beneficial biological activity for the plant [18].

Interestingly, there were no statistical significant differences between the three shading regimes. It is shown here for the first time that the content of PD and HG for each cultivar tested is not affected by shading. While shading could be important for plant growth [17], it will not influence PD or HG content as a quality parameter.

Pooling data for cultivar and lighting regime by calculating the mean over all three cultivars and shading scenarios (Figure 4E,F) showed the same dependency of PD and HG content in younger versus older leaves as observed for single cultivars under different lighting regimes. To compare PD and HG content, it is a prerequisite to investigate the same leaf age. In further studies, we used the upper leaves, because the highest PD and HG content would reveal differences more easily. The results on differences in HG, PD and DHCs between cultivars can be found in Figure 5, with columns of the same coloring representing different leaf ages, from the upper third leaves (left column) to leaves taken from the middle third (middle column) and lower third (right column).

PD content varied significantly between the three cultivars, with 'Amagi Amacha' containing the highest amount of PD in leaf dry matter under partial and full shade conditions in 2018 and partial shade in 2019 (Figure 5). The mean PD content over all shading scenarios and years was 4.2% compared to 3% in 'Oamacha' and 1.6% in 'Odoriko Amacha'. The PD within a cultivar (regardless of lighting treatment) only displayed significant differences between the years in 'Amagi Amacha'. PD content shows to be mainly influenced by cultivar. Using *H. macrophylla* subsp. *serrata* as a feedstock for PD or tea, the cultivar selection seems more important than the managing factors. Figure 5 illustrates the PD content in relation to cultivar, sun exposure and year when harvested in mid-September.

Significant differences in HG content in leaf dry matter were only observed between the three cultivars or year but not for shading scenarios. *H. macrophylla* subsp. *serrata* 'Odoriko Amacha' yielded the highest HG content of the three cultivars (2.9 $\pm$ 0.58%), with significantly higher HG in 2019 (3.16 $\pm$ 0.44%) compared to 2018 (2.61 $\pm$ 0.73%) when pooled over all treatments. 'Amagi Amacha' seems to convert HG into PD at a very high rate, as only 0.1 $\pm$ 0.04% of HG was found in leaf dry matter, in combination with high PD content (Figure 5). Over all treatments and years, 'Amagi Amacha' yielded significantly lower HG than both other cultivars. Additionally, the results presented show that higher amounts of HG or PD do not influence total DHC content, as the high HG cultivar 'Odoriko Amacha' and high PD cultivar 'Amagi Amacha' only expressed significantly different DHC content under no shade conditions in 2019. In 2018, shading did not affect PD, HG and DHC content. HG, PD and DHC content related to cultivar, shading treatment and year are illustrated in Figure 5.

3.2. Fresh Biomass

The total biomass of hortensia cultivars is shown in Figure 6. Pooled over all treatments, 'Amagi Amacha' yielded significantly less biomass in 2018, whereas no statistical differences were found in 2019, except for 'Amagi Amacha' with no shading, which differ only to 'Odoriko Amacha' with full shading. Differences in year might be due to differences in plant material, provided by the commercial breeder. Environmental effects seem neglectable based on the climate data presented in Figures 1 and 2. The combination of cultivar and sun exposure showed no clear impact of radiation level for the two years. This is in accordance with a study on colored shading nets for H. macrophylla, where no significant effects on plant height (cm) and LAI from cultivation under black, red and blue shading were found for most of the cultivars tested [51]. For 'Amagi Amacha', full shade conditions seem optimal for higher biomass; for 'Oamacha' and 'Odoriko Amacha', no clear advice has been given. In 2018, no shade cultivation expressed the highest biomass, while in 2019, (partial) shade yielded higher biomass. For high-yield production, older plants would be used. Therefore, plant growth in this young state of plant development seems unreasonable as a decision parameter for cultivar selection or a final determination of light management. Light exposure over the duration of the experiments (measured in mean hours of sunshine) did not reveal significant differences between 2018 and 2019 and is therefore not responsible for differences between years.

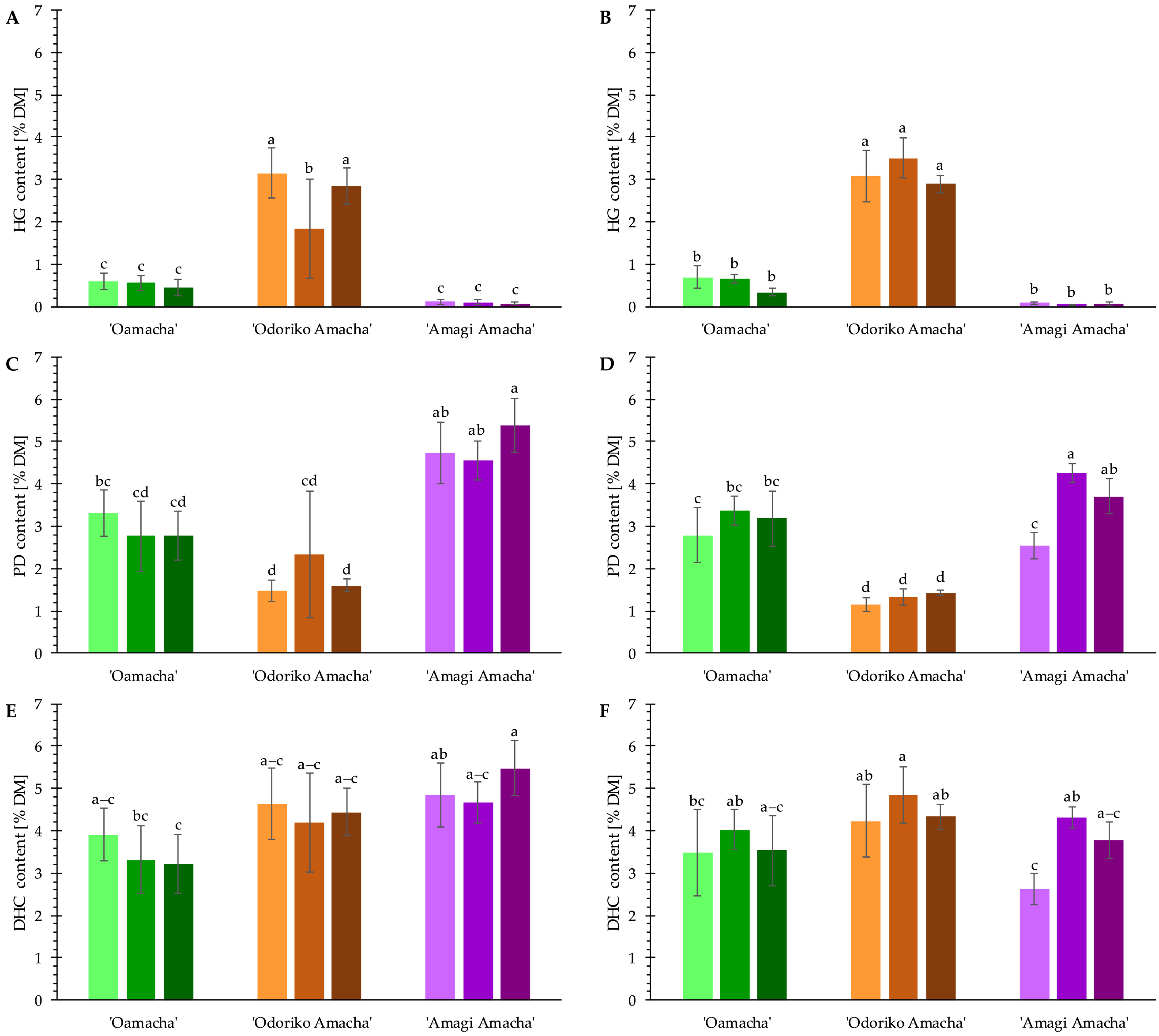


Figure 5. Content (%) of (**A**,**B**) hydrangenol (HG), (**C**,**D**) phyllodulcin (PD) and (**E**,**F**) dihydroisocoumarin (DHC) in leaf dry matter of *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') in two different years ((**A**,**C**,**E**): 2018 and (**B**,**D**,**F**): 2019) at the end of two growing periods under three different light conditions (bright coloring: no shade, light coloring: partial shade, dark coloring: full shade). Significant differences within a year and constituent calculated by ANOVA and Tukey HSD (n = 5, α = 0.05) are indicated by letters (a–d). ANOVA tables can be found in the Supplementary Materials Table S6.

Different content of the DHCs (HG and PD) in *Hydrangea macrophylla* subsp. *serrata* presents challenges in yield estimation. As a function of biomass and DHC content, further research is needed to estimate the possible PD yield per plant or under practical conditions in the field. Additionally, further understanding of plant age and growth stage on PD content is needed to optimize harvest dates and PD yield over the growing cycle in *H. macrophylla* subsp. *serrata* cultivation management by inducing branches to increase the number of young leaves per plant or other measures such as targeted fertilization to increase PD. Branching can be induced by pruning or the application of dikegulac sodium, benzyladenine or ethephon [52]. Combinations of pinching or pruning and dikegulac sodium influence not only branch numbers but also leaf area [53]. Therefore, a clear management of branch-inducing techniques is necessary to achieve the maximum leaf biomass of young *H. macrophylla* subsp. *serrata* leaves. Additionally, further analysis is needed to validate the yield increase of branch-inducing treatments for total PD yield per plant. Phytotoxicity and residues of chemical treatments might be an issue for plant health and product processing. The phytotoxicity of Augeo, Configure and Florel induced damage in *Hydrangea macrophylla* 'Merritt's Supreme' and 'Nikko Blue', as well as *H. paniculata* 'Limelight', and the plants recovered from six weeks after treatment [54].

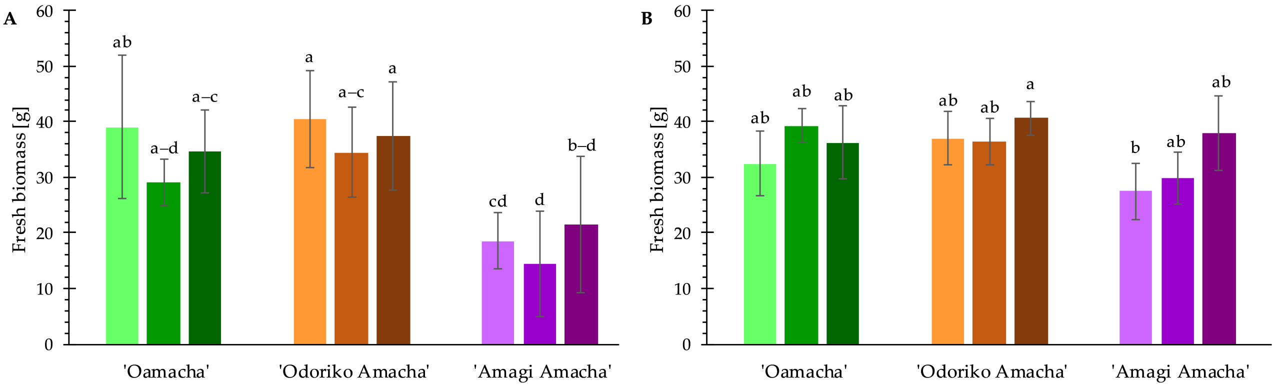


Figure 6. Total fresh biomass (g) of *H. macrophylla* subsp. *serrata* (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') in two different years ((**A**): 2018 and (**B**): 2019) at the end of two growing periods under three different light conditions (bright coloring: no shade, light coloring: partial shade, dark coloring: shade). Significant differences within a year calculated by ANOVA and Tukey HSD (n = 5, α = 0.05) are indicated by letters (a–d). Mean $\pm$ standard deviation values can be obtained via the Supplementary Materials Table S3.

3.3. Plant Phenotyping

High amounts of radiation can lead to a typical leaf browning in *Hydrangea* [17]. To characterize this effect for the *H. macrophylla* subsp. *serrata*, leaf color measurements were performed. These leaf color measurements revealed differences between cultivars (Figure 7). 'Odoriko Amacha' only manifested significant differences between the first and last measurements in 2018. Differences in 'Oamacha' were the highest of the three cultivars, with significant differences under full sun and partial shade in both years. 'Amagi Amacha' showed significant differences in all three light scenarios in 2018, while differences in 2019 were only significant under partial shade conditions. Still, most of the cultivar-shading combinations revealed tendencies of decreasing hue angles over the time of the trial (Figure 7).

This shift can be related to a typical leaf browning reaction of hortensias to high levels of sun exposure that lead to wilting [21]. Sun tolerance of *Hydrangea macrophylla* has been shown to be cultivar dependent [55]. Therefore, 'Odoriko Amacha' seems to be the genotype that copes with UV stress best, as differences were only significant in 2018, while 'Amagi Amacha' and 'Oamacha' expressed lower adaptation. This color shift can also be seen using the vegetation indices CRI and ARI (see Figure 9), which are related to the redness of the leaves. The change in hue angle from the first to last measurement over all treatments and years was the lowest in 'Amagi Amacha' (11.01° under no shade conditions in 2018) and the highest in 'Oamacha' (34.36° under no shade conditions in 2018). The results indicate that differences depend on genotype and light intensity. Differences by year were not based on hours of sunshine, as no significant differences were observed (Figure 1).

Leaf color changes are derivable from reflection spectra by visual interpretation and the calculation of different vegetation indices. The reflection spectra expressed differences between cultivars as well as between shading scenarios. Additionally, differences from the first to last day of measurements could be observed. An overview of the reflectance spectra from *H. macrophylla* subsp. *serrata* is presented in Figure 8. Because the interpretation of pure reflectance spectra regarding multiple plan parameters is not trivial, multiple VIs derived from the reflectance spectra were analyzed. Generally, the reflectance response curves of the three cultivars showed that the effect depends on cultivar, year and shading regime. The observed differences were lower in 2019 in comparison to 2018. For 'Odoriko

Amacha', the differences between the first and last day of measurements were more pronounced than for the other two cultivars, especially in 2018. Higher reflectance could be observed around 550 nm (Figure 8A2,B2,C2) at the last day in 2018. This shift was also observed for 'Oamacha' and 'Amagi Amacha' but to a lesser extent.

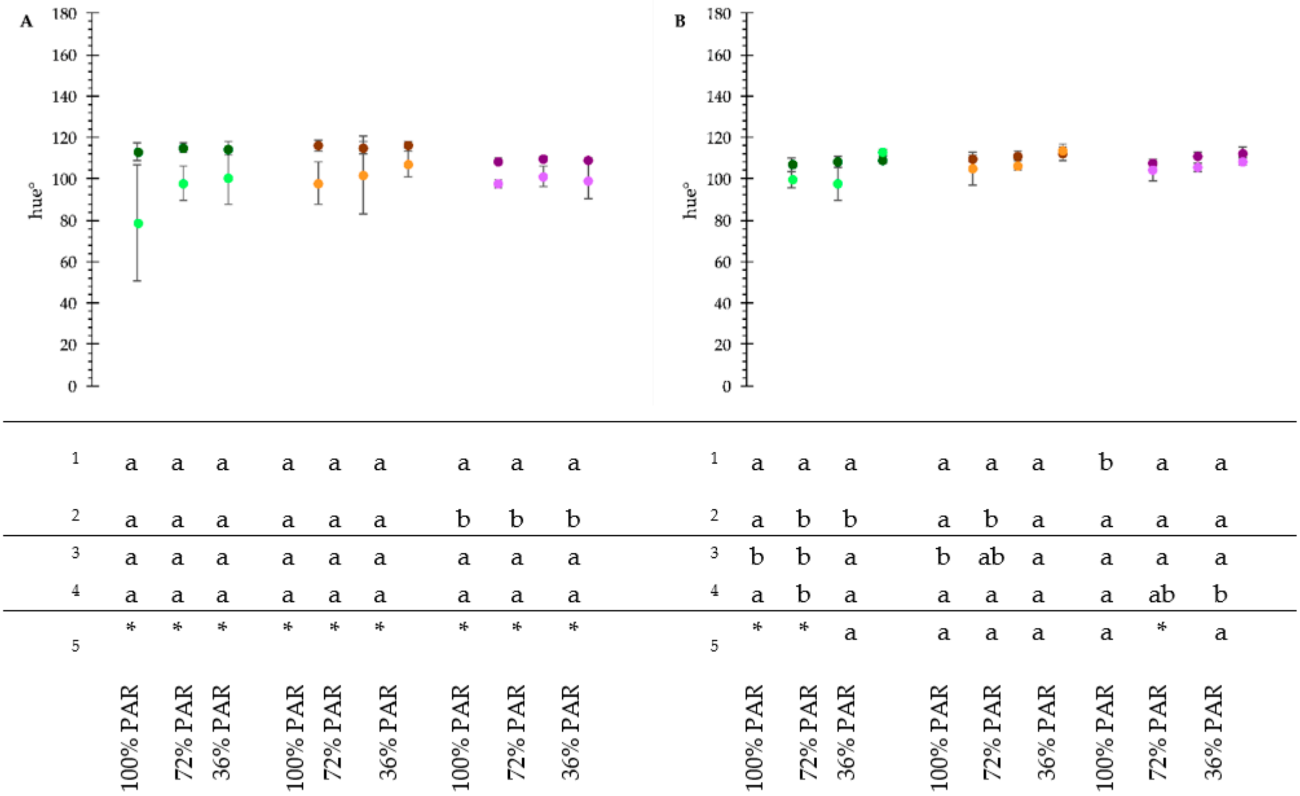


Figure 7. Hue angle of three different *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') at first (dark coloring) and last (light coloring) day of measurements in two cultivation periods ((**A**): 2018; (**B**): 2019). Shading regimes are indicated by PAR values (100% PAR: no shade, 72% PAR: partial shade, 36% PAR: full shade). Significant differences are highlighted by letters. Differences within cultivar at first (1) and last (3) day of measurements and differences within light regime at first (2) and last day (4) of measurements were calculated by ANOVA and Tukey HSD (n = 5, α = 0.05). Differences between first and last day of each treatment (5) were calculated by Student's t-test with significant differences marked by asterisk (*). ANOVA tables can be found in the Supplementary Materials Table S6.

The Anthocyanin Reflectance (ARI1) as an indicator for leaf redness illustrates significantly higher values under full sun exposure in 'Oamacha' in 2018 and 'Amagi Amacha' in 2019 (Figure 9A,B). Anthocyanins can function as a reducing factor for photoinhibition and leaf damage [56]. Therefore, the photoinduction of anthocyanins via UV, Vis and far-red wavelengths has been described [57]. In addition to the photoinhibition-related effects, nutrient deficiencies in phosphorus (P) or nitrogen (N) are indicated by purpling due to anthocyanin accumulation [56].

The CRI 1 as an indicator for carotenoids only showed significant differences between no shade and (partial) shade in 'Oamacha' (Figure 9C,D; Table S5). It was shown that carotenoids can be an indicator for high irradiance adaptation of leaves [58]. Therefore, 'Oamacha' seems to adapt to high sun exposure better than 'Amagi Amacha' and 'Odoriko Amacha' when using CRI 1 as an indicator.

Chlorophyll and the general greenness of *Hydrangea macrophylla* subsp. *serrata* leaves were estimated using the vegetation indices MCARI1 (Figure 9G,H) and the Greenness Index, G (Figure 9E,F). While differences in MCARI1 were not significant in 2019, 2018 displayed significantly lower values for 'Oamacha' under full sun conditions in comparison to the other cultivars and treatments at the end of the experiment (Table S5). The lowest values for 'Amagi Amacha' were found under partially shaded conditions. Higher chlorophyll-related index values in 'Amagi Amacha' under full shade conditions compared to partial shade were also measured by the Greenness Index (G). In 2018, the difference of full shade to no shade was not significant, while in 2019, significant differences were observed (Table S5). While not significant in 2019, 2018 showed significantly higher chlorophyll content under (partially) shaded conditions compared to full sun exposure, with full shade reaching the highest values. High amounts of UV radiation have negative effects on chlorophyll due to higher concentrations of carotenoids and anthocyanin [59–61].

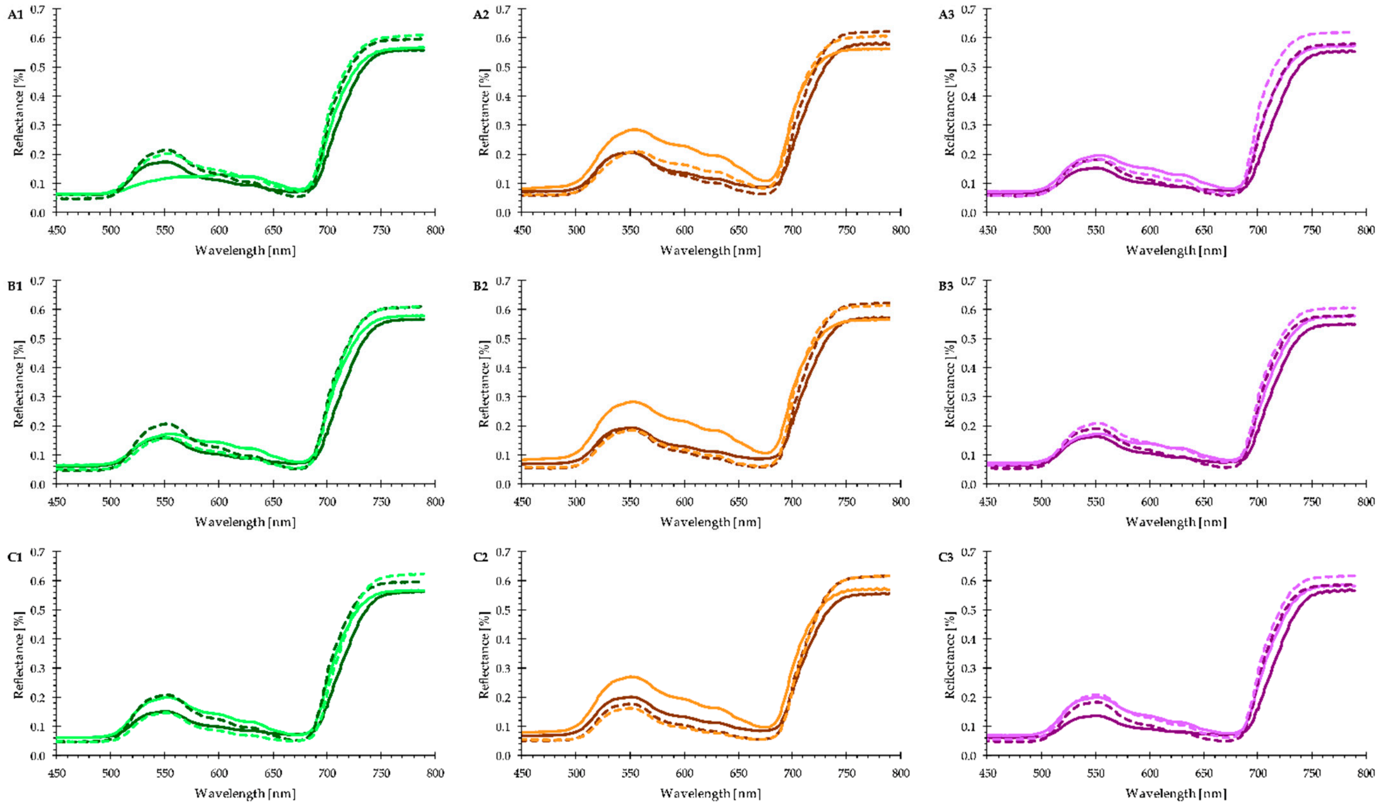


Figure 8. Reflectance spectra of three different *H. macrophylla* subsp. *serrata* cultivars (**1**: 'Oamacha', **2**: 'Odoriko Amacha', **3**: 'Amagi Amacha') under three different shading regimes ((**A**): no shade, (**B**): partial shade, (**C**): full shade). Full lines represent 2018 and dotted lines 2019, with dark coloring representing first and light coloring representing last day of measurements. Full data of reflectance spectra can be found in the Supplementary Materials Table S4.

Different shading regimes influencing not only stress-related radiation (UV-B) but also effects on the photosynthesis of *H. macrophylla* subsp. *serrata* due to reduced PAR were expected. Light use as indicated by PRI increased significantly with each shading level in 2018 in 'Oamacha' (Figure 10A,B, Table S5). Significant increases were measured under full sun conditions compared to under full shade conditions in 'Amagi Amacha', and under (partial) shade conditions compared to full sun conditions in 'Odoriko Amacha' in 2018. In 2019, full shade yielded a higher PRI compared to full sun in all *H. macrophylla* subsp. *serrata* cultivars. PRI can be used as a proxy for water stress. This is only applicable under controlled conditions under low or moderate stress levels [62]. Therefore, the use of PRI as a proxy for water stress might be difficult to interpret in the presented experimental setup. PRI can be interpreted as an indicator for light-use efficiency (LUE) with an R^2 of around 0.6 for leaves as well as full canopy [63]. Generally, the PRI is a reliable proxy for photosynthetic efficiency [36]. In conclusion, higher amounts of shading seem to increase photosynthetic efficiency in the experiments. This effect should be evaluated in further studies over a complete growing season to investigate if this leads to statistical differences over a growing cycle as well as yield differences over the span of the cultivation.

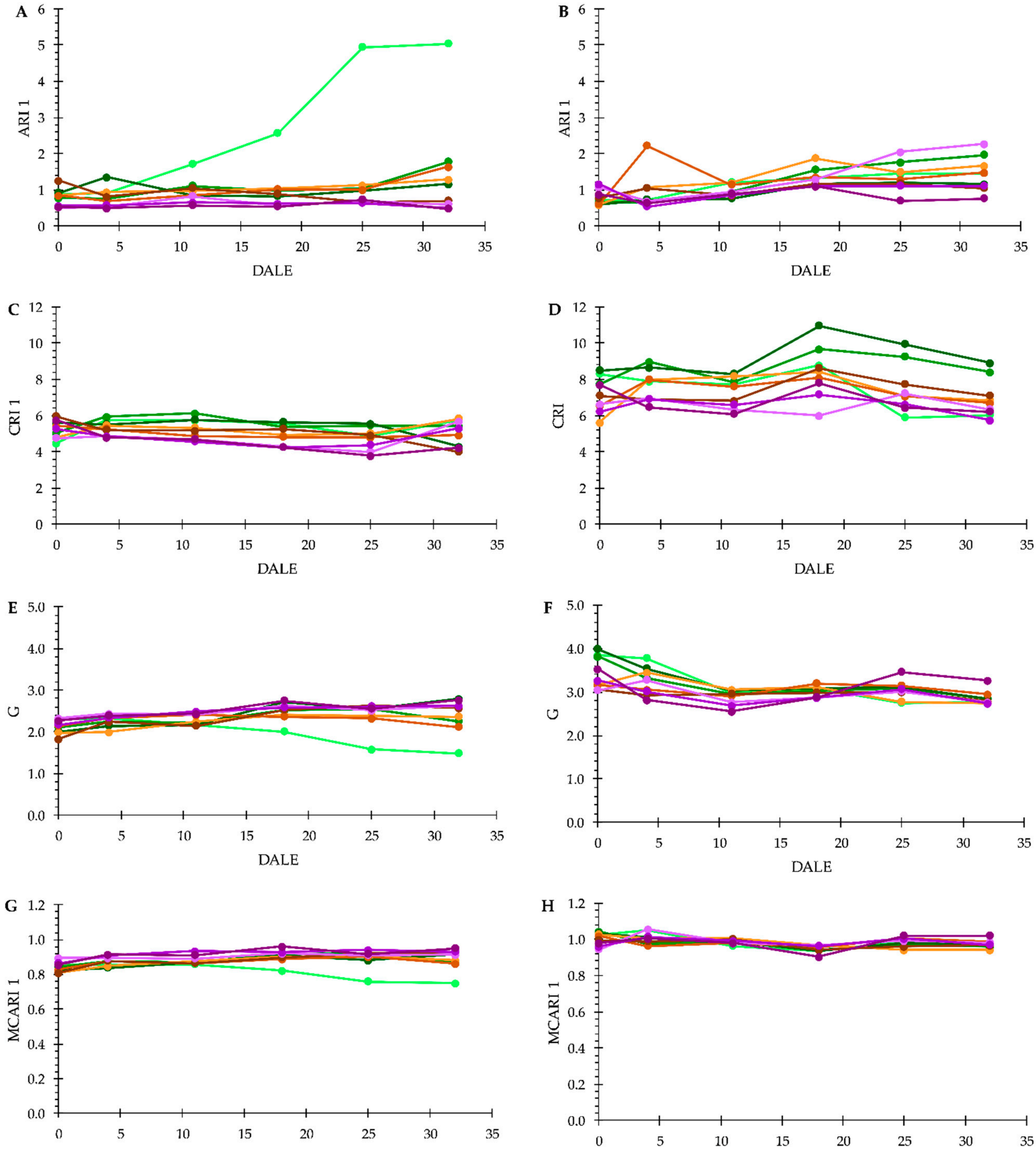


Figure 9. Pigment-based vegetation indices for *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') under different shading conditions (dark coloring: full shade, medium coloring: partial shade, light coloring: no shade) in 2018 (**A**,**C**,**E**,**G**) and 2019 (**B**,**D**,**F**,**H**). Full display of significant differences is reported in the Supplementary Materials Table S5.

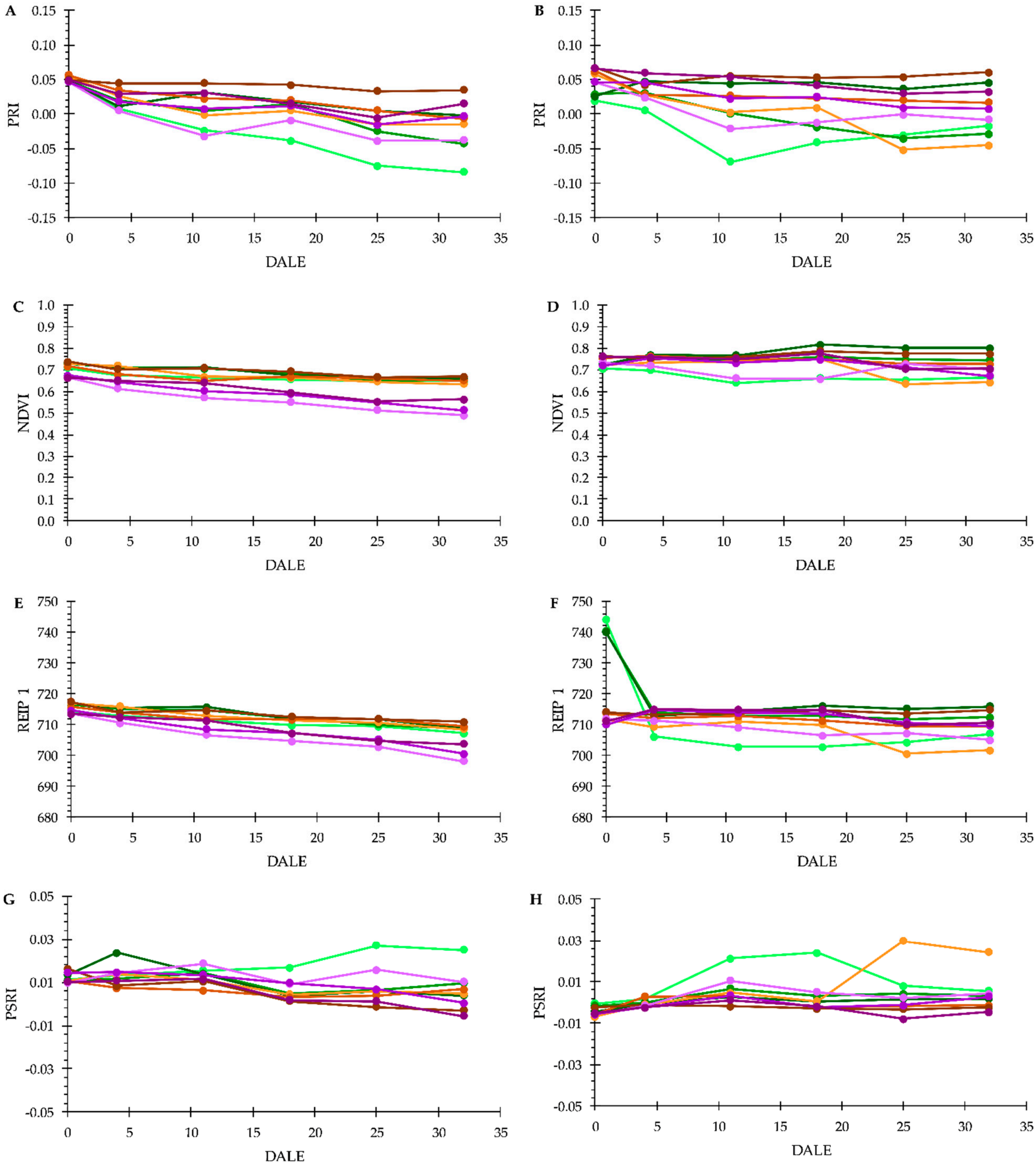


Figure 10. Performance-based vegetation indices for *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') under different shading conditions (dark coloring: full shade, medium coloring: partial shade, light coloring: no shade) in 2018 (**A**,**C**,**E**,**G**) and 2019 (**B**,**D**,**F**,**H**). Full display of significant differences is reported in the Supplementary Materials Table S5.

The NDVI as a general indicator for plant performance revealed general tendencies that differ within the two years 2018 and 2019 (Figure 10C,D). In 2018, 'Amagi Amacha' expressed the lowest values of the three cultivars with a decrease over time in all three treatments. Sun exposure had no significant effect. In 2019, no significant differences were observed between the cultivars. Still, significant differences resulting from full sun exposure in comparison to full shade were found in 'Oamacha' and 'Amagi Amacha' at the last day of measurements (Table S5). NDVI as an indicator for plant health and productivity indicates no differences by cultivar but showed a significant decrease in the no shade scenario in 2019. With an increase in UV-B, yield of crops is reduced [64]. High amounts of UV-B radiation result in lower chlorophyll content [65]. This negative effect of higher solar radiation was confirmed for *H. macrophylla* subsp. *serrata*, as NDVI values were significantly lower under no shade conditions in 2019 and tendencies of lower values in 2018 were found. Therefore, (partial) shading is advised to reduce the negative effects of solar radiation on plant performance.

The position of the REIP is determined by the amount of chlorophyll [66,67]. In addition, the red edge is affected by severe water deficiency [67]. Similar to the other greenness and chlorophyll measurements (e.g., SPAD or greenness-related VIs), this chlorophyll assessment allows for an estimation of the nitrogen status [39]. The Red Edge Inflection Point (REIP 1) yielded no significant differences between light intensities within a cultivar in 2018 (Figure 10E). In 2019, all three *H. macrophylla* subsp. *serrata* cultivars yielded longer wavelengths for the inflection point under full shade in comparison to full sun exposure (Figure 10F; Table S5). For 'Odoriko Amacha', the differences between partial shade and no shade were also significant.

UV-B radiation has the potential to influence leaf age and senescence [65]. Leaf senescence (as indicated by PSRI) defined by chlorophyll, leaf protein and nitrogen loss, as well as decreased photosynthesis [68], presents the counterpart of NDVI. The mean PSRI over all cultivars increased significantly under full sun exposure compared to full shade conditions toward the end of the field trial in 2018 (Figure 10G; Table S5). In 2019, a significant increase in senescence was observed in 'Amagi Amacha' (Figure 10H; Table S5). PSRI in the no shade scenario of 'Oamacha' on DALE 11 and 18 was not significantly different from partial shade and full shade conditions, while tendencies of higher PSRI values were obvious. The same negative effects of UV-B radiation as shown for NDVI apply for PSRI. The combination of NDVI and PSRI supports the hypothesis that cultivars react differently toward sun exposure. Based on both indices, shading seems to provide benefits, while not being necessary for a successful cultivation.

4. Conclusions

Shading and light quality have been described to be necessary for the cultivation of *Hydrangea* to avoid excessive wilting [21] and in consequence preventing negative impacts on yield parameters. This could affect phyllodulcin (PD) content as well as PD yield per area in *H. macrophylla* subsp. *serrata*. In the present study, we compared the effects on three different cultivars. It was shown that cultivar determines the PD content, with 'Amagi Amacha' showing the highest content followed by 'Oamacha' and 'Odoriko Amacha'. For HG, the order was reversed. Therefore, cultivar selection is a prerequisite in *Hydrangea* cultivation to achieve high PD yield per area.

Leaf age was the second factor determining PD content. Younger plant parts have higher PD than older plant parts. This effect of leaf age on PD content was similar for all cultivars. It can be concluded that leaf age has to be considered when harvesting *Hydrangea* to optimize PD yield per area in general, but it is not a factor for cultivar selection.

It could be shown that shading had little influence on PD content. Only for 'Amagi Amacha' could higher PD content be found by shading in 2019. However, shading could have beneficial effects on plant performance during cultivation to mitigate light stress. In this context, the color and hyperspectral analysis showed a more complex response of the three cultivars, which have to be investigated in more detail in the future. Understanding the differences in the pattern of vegetation indices (e.g., NDVI or ARI where 'Amagi Amacha' could be distinguished) could be important for management strategies to predict plant performance.

The results of this study showed that the selection of cultivar is more important than the management factor (in this case shading levels) for DHC production. Still, the analysis of vegetation indices revealed a higher stress response in non-shaded *H. macrophylla* subsp. *serrata*. The effect of plant age and growth stage has to be considered as a factor in perennial crops for genotype selection, as the cultivars used in this study could develop differently over multiple growing seasons.

Supplementary Materials: The following data are available online in one single file at https://www.mdpi.com/article/10.3390/agronomy11091743/s1, Table S1: Irradiation data; Table S2: Climate data; Table S3: Reflectance spectra of *H. macrophylla* subsp. *serrata* cultivars under different shading regimes at first and last day of measurements; Table S4: Vegetation indices over the course of the experiment; Table S5: Fresh biomass of *H. macrophylla* subsp. *serrata* cultivars; Table S6: ANOVA tables; Table S7: multivariate analysis for year 2018; Table S8: Detailed ANOVA for year 2018.

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Data Availability Statement: The data presented in this study in the tables, figures and Supplementary Materials are available. Additional raw data is available on request from the corresponding author.

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