Institut für Nutzpflanzenwissenschaften und Ressourcenschutz (INRES)

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Cultivation and utilization of *Hydrangea macrophylla* subsp. *serrata* as feedstock for dihydroisocoumarins and its quality-assessment through non-invasive phenotyping

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Summary

New and innovative bio-based products of today are regularly inspired by a long-known and traditional use of plants. One such example is the taste modifying phyllodulcin (PD), a dihydroisocoumarin (DHC) only present in tea-hortensias (*Hydrangea macrophylla* subsp. *serrata*). For industrial applications of DHCs like PD or its precursor hydrangenol (HG) in the field of innovative taste-modifying solutions or medicine, establishment of cultivation on a large scale and selection of best cultivars are needed. Therefore, a targeted quality-assessment towards DHC content and plant performance to optimize yield is imperative.

Experiments on tea-hortensia cultivation at INRES – Renewable Resources and Campus Klein-Altendorf, Germany revealed that while ornamental *Hydrangea* benefits from shading, sun exposure does neither influence biomass accumulation nor HG and PD in tea-hortensias. Still, higher symptoms of stress under high sun exposure were detected via non-invasive phenotyping. This trial was the first to indicate that HG and PD are genetically predetermined but not significantly affected by external stressors except for seasonal changes.

Investigations of 12 sub-experiments within the second major experiment were used to develop a chemometric marker for the conversion of HG into PD. Analogue to Vegetation Indices, the chemometric Normalized Difference Phyllodulcin Index (cNDPI) could be a reliable and accurate tool for quantifying HG-into-PD conversion with utilization as a marker in plant breeding.

In order for farmers to identify optimized harvest dates, a third experiment investigated the possibilities of non-invasive measurements to quantify HG and PD contents via hyperspectral modeling. While highly accurate models on a single-leaf scale were not obtained, approximations on a field-scale showed great potential.

The achieved results allow for a quality-assessment via non-invasive methods. Thus, environmental and management-related factors can be evaluated while parallelly estimating the quality of plant material in regard to PD content and synthesis (cNDPI). In the future, the combination in one hand-held spectrometer could bring all three compartments together to provide a holistic assessment of plant traits for farmers and breeders working with tea-hortensias.

Zusammenfassung

Lang bekannte und traditionelle Verwendungen von Pflanzen dienen heutzutage oftmals als Inspiration für neue und innovative biobasierte Produkte. Ein Beispiel hierfür ist das geschmacksmodulierende Phyllodulcin (PD), ein Dihydroisocumarin (DHC), das nur in Teehortensien (*Hydrangea macrophylla* subsp. *serrata*) vorkommt. Für industrielle Anwendungen von DHCs wie PD oder seinem Vorläufer Hydrangenol (HG) im Bereich innovativer geschmacksverändernder Lösungen oder der Medizin sind die Etablierung eines Anbaus in großem Maßstab sowie die Auswahl der geeignetsten Kultivare erforderlich. Daher ist eine gezielte Qualitätsbewertung im Hinblick auf den DHC-Gehalt und die Performance der Pflanzen zur Optimierung des Ertrags unerlässlich.

Experimente zum Anbau von Teehortensien am INRES – Nachwachsende Rohstoffe und Campus Klein-Altendorf, Deutschland, zeigten, dass die Zierhortensie von Beschattung profitiert, während die Sonneneinstrahlung weder Biomasseakkumulation noch HG und PD der Teehortensien beeinflusst. Dennoch wurden mittels nicht-invasiver Phänotypisierung stärkere Stresssymptome bei starker Sonneneinstrahlung festgestellt. Dieser Versuch war der erste, der darauf hindeutet, dass HG und PD genetisch determiniert sind und durch externe Stressfaktoren, mit Ausnahme von saisonalen Veränderungen, nicht signifikant beeinflusst werden.

Die Untersuchungen von 12 Unterexperimenten innerhalb des zweiten Hauptversuchs wurden genutzt, um einen chemometrischen Marker für die Umwandlung von HG in PD zu entwickeln. Analog zu Vegetationsindizes könnte der chemometric Normalized Difference Phyllodulcin Index (cNDPI) ein zuverlässiges und genaues Instrument zur Quantifizierung der Umwandlung von HG in PD sein und als Marker in der Pflanzenzüchtung eingesetzt werden.

Damit Landwirte optimale Erntetermine ermitteln können, wurden in einem dritten Versuch die Möglichkeiten nicht-invasiver Messungen zur Quantifizierung des HG- und PD-Gehalts mittels Modellierung basierend auf Hyperspektraldaten untersucht. Während präzise Modelle im Einzelblattmaßstab nicht erreicht wurden, zeigten Abschätzungen für den Feldmaßstab großes Potenzial.

Die erzielten Ergebnisse ermöglichen eine Qualitätsbeurteilung mittels nicht-invasiver Methoden. So können umwelt- und managementbezogene Faktoren bewertet werden, während gleichzeitig die Qualität des Pflanzenmaterials im Hinblick auf PD-Gehalt und -Synthese (cNDPI) abgeschätzt werden kann. In Zukunft könnte die Kombination in einem Handspektrometer alle drei Bereiche zusammenführen, um Landwirten und Züchtern, im Bereich der Teehortensien, eine ganzheitliche Bewertung von Pflanzenmerkmalen zu ermöglichen.

Abbreviations

2D	Two-Dimensional
3D	Three-Dimensional
Al	Aluminum
AMP	Adenosin Monophosphate
ANOVA	Analysis of Variance
ARI1	Anthocyanin Reflectance Index 1
BMEL	Federal Ministry of Food and Agriculture
C	Carbon
c	Calibration Set
Ca	Calcium
CET	Central European Time
cNDPI	chemometric Normalized Difference Phyllodulcin Index
CO_2	Carbon Dioxide
CRI1	Carotinoid Reflectance Index 1
CT	Computed Tomography
DALE	Days After Light Exposure
DHC	Dihydroisocoumarin
DM	Dry Matter
DOY	Day Of Year
E	Environment
e_i	Residuals
F_1	First filial generation
Fe	Iron
FWHM	Full-Width at Half-Maximum
G	Greenness Index
H	Hydrogen
h°	Hue Angle
HG	Hydrangenol
HPLC	High Performance Liquid Chromatography
IR	Infrared Radiation

K	Potassium
LAI	Leaf Area Index
LUE	Light Use Efficiency
MCARI1	Modified Chlorophyll Absorption in Reflectance Index 1
Mg	Magnesium
MLR	Multiple Linear Regression
Mn	Manganese
MRI	Magnetic Resonance Imagers
N	Nitrogen
n	Number of Replicates or Samples
NDBaI	Normalized Difference Bareness Index
NDVI	Normalized Difference Vegetation Index
NDWI	Normalized Difference Water Index
NIPALS	Non-Linear Iterative Partial Least Squares
NIR	Near Infrared Radiation
NPCI	Normalized Pigment Chlorophyll Index
NPQI	Normalized Phaeophytinization Index
O	Oxygen
P	Phosphorus
p	Prediction Set
PAR	Photosynthetic Active Radiation
PD	Phyllodulcin
PET	Positron Emission Detectors
PKS	Polyketide Synthase
PLS	Partial Least Square
PLSR	Partial Least Square Regression
PPFD	Photosynthetically Active Photon Flux Density
PRESS	Predicted Residual Sum of Squares
PRI	Photochemical Reflectance Index
PRI	Photochemical Reflectance Index
P-Spline	Penalized Basis Spline
PSRI	Plant Senescence Reflectance Index

QTL	Quantitative Trait Locus
r	Correlation Coefficient
R^2	Coefficient of Determination
REIP1	Red-Edge Inflection Point 1
RMSE	Root Mean Square Error
S	Sulfur
S1–S8	Supplementary Material 1–8 for Chapter 2
SD	Standard Deviation
SE	Sub-Experiment
SG	Savitzky-Golay
SIPI	Structure Insensitive Pigment Index
SNV	Standrd Normal Variate
SWIR	Sort Wavelength Infrared
$_{total}$	Overall Model
UAV	Unmanned Aerial Vehicle
UPLC	Ultra Performance Liquid Chromatography
UV	Ultra Violet
UV-A	Radiation from 380–315 nm
UV-B	Radiation from 315–280 nm
UV-C	Radiation from 280–100 nm
$_{v}$	Validation Set
VI	Vegetation Index
VIP	Variable Influence on Projection
VIS	Visual Light Spectrum
VOC	Volatile Organic Compounds
$\mathbf{X}$	Predictors
$\mathbf{y}$	Response
y_i	Measured Values
$\hat{y}_i$	Predicted Analyte Concentration

Chapter 1 – General Introduction

Over time, numerous innovative renewable resources were researched and developed. Some of those plants were formerly used as ornamental plants or were not cultivated at all, as only wild forms were collected. One of these innovative renewable resources is the “tea-hortensia” (*Hydrangea macrophylla* subsp. *serrata*) that gained industrial interest because of their secondary metabolites hydrangenol (HG) and phyllodulcin (PD). For the successful cultivation of tea-hortensia, profound knowledge on physiological effects of cultivation is imperative. Therefore, non-invasive plant phenotyping can be beneficial to quantify plant responses. This general introduction builds the foundation for the in-depth scientific details presented in the following chapters and ends on the research objectives of this thesis.

1.1 Development towards new renewable resources

Renewable resources are defined as crops that are used for purposes other than food production (Eggersdorfer et al., 1992). They recently gained more attention as a mitigation strategy in regard to climate change (Owusu and Asumadu-Sarkodie, 2016), even though some critique about social pressure in regard to renewable resources can be put forward (Tavoni et al., 2012). Still, plants that are used as renewable resources are impacted by climate change as well (Gernaat et al., 2021). In addition to a sole use of whole plants, plant compartments like leaves can be used as packing materials (Kora, 2019). New and innovative ways to utilize known plants is a key field of research towards new plants as renewable resources. One prominent example is *Miscanthus*, a perennial C_4 grass that can be used in material production (Moll et al., 2020). Before its usage as a renewable resource, *Miscanthus* is long known as an ornamental plant with low input requirements, a broad climate tolerance, and a multitude of different cultivars (Dougherty et al., 2014). The way of plants being used as ornamentals and later finding use as renewable resources is a common one. Maybe the most prominent examples of plant species that are being used as ornamentals and renewables are rose (*Rosa* L.), poppy (*Papaver* L.), and crocus (*Crocus sativus* L.). The latter is prominently known as feedstock for saffron, the red stigma of the crocus plant. Saffron is the most expensive spice worldwide and known as the “king of the spices” (Cardone et al., 2020). Besides that, medicine, dye, and perfume were also products made of saffron, but nowadays the food and spice utilization is prioritized (Basker and Negbi, 1983) with the largest area of cultivation being in Iran, Spain and India (Negbi, 2005). Poppy is one of the oldest medicinal plants, prominently used for opium production. Still, poppy contains a large number of interesting constituents and is used as an ornamental plant (Duke, 1973) while content and composition of *Papaver somniferum* oil and seeds are highly influenced by variety used (Luhmer et al., 2021). Roses as renewable resource are mainly used for oil and seeds (Özcan, 2002) and are rich in essential oils, as well as phenolics, flavonoids, and carotenoids (Ghazshazi et al., 2010). The usage as feedstock for essential oils puts forward some key challenges, as essential oil content and composition are heavily affected by external effects (Baydar and Baydar, 2005).

Aromatic plants are a distinct group within the renewable resources. Other names for aromatic plants are "herbs" or "spices" with some examples being anise (*Pimpinella anisum*) basil (*Ocimum basilicum*) oregano (*Origanum vulgare*) and rosemary (*Salvia rosmarinus* or synonymously *Rosmarinus officinalis*) (Christaki et al., 2012) and are also used as ornamentals (Bertoli et al., 2010; Rinaldi et al., 2014). Besides their usage in the food sector or ornamental plants, aromatic plants fulfill a wide range of industrial applications, ranging from essential oil production, dye and colorant production, cosmetics, and plant protection products or the use as medicinal plants (Lubbe and Verpoorte, 2011). Aromatic plants are often closely linked to medicinal plants (Rao et al., 2004; Bogers et al., 2006; Lubbe and Verpoorte, 2011). The medicinal use results from the potential use of extracts and essential oils from aromatic plants (Christaki et al., 2012) and could even provide potential benefits against COVID-19 (Mani et al., 2020). To advance towards new genotypes in the spectrum of aromatic and medicinal plants, multiple strategies are applicable. First, the natural biodiversity with its natural mutations sets the foundation and is followed by targeted selection. Crossing (inter- and intraspecific) and polyploidization are profound tools to improve plant traits and therefore plant performance. Lastly, genetic engineering can be applied to generate new genotypes in aromatic and medicinal plants (Bernáth, 2002). For high-quality production, intercropping and agroforestry systems can be beneficial for the cultivation of aromatic and medicinal plants and simultaneously being environmentally friendly in comparison to monoculture (Rao et al., 2004). One of the environmental benefits is biodiversity, that is irreversibly linked to functional and sustainable agroecosystems (Altieri, 1999) and the interaction of pollinator-plant networks (Blüthgen and Klein, 2011).

As mentioned above, plant-based aroma and taste are important characteristics for the food industry. A 2015 review article illustrated the importance of medicinal and aromatic plants in poverty reduction in developing countries due to high product value. This high value leads to medicinal and aromatic plants being highly interesting in business developments (Kala, 2015) as their return on investment is much higher than for traditional crops (Mittal and Singh, 2007). A prominent functional component that fulfilled the new arising demand of plant-based sweeteners introduced into the food industry was *Stevia rebaudiana* (Savita et al., 2004) with its steviol glycosides (Tanaka, 1982). Stevia as a sugar substitute for diabetics is safe, as blood sugar levels are not affected. Additionally, there are no side effects that can be attributed to artificial sweeteners. As other aromatic plants, *Stevia* possesses multiple positive side effects that make it interesting for herbal medicines (Goyal et al., 2010). As *Stevia* gained popularity, further optimization was carried out by using mutation, polyploidy, and heterosis breeding to improve plant quality and expression of desired plant traits and bioactive functions (Yadav et al., 2011). Generally, consumer acceptance of *Stevia*-based products is high when being informed about it (Kamarulzaman et al., 2014; Saharudin et al., 2020). Still, the acceptance of agricultural products is of mayor importance, as food production is more and more seen as a black box for consumers (Siegrist and Hartmann, 2020). On the other hand, chocolate-milk sweetened only with stevia in comparison to sucrose only and sucrose-stevia mixtures showed that acceptance of stevia-only sweetened chocolate milk was significantly lower (Bordi et al., 2016). A bitter and licorice after taste of *Stevia*-derived sweeteners is one key limitation for its usage in beverages and other products (Bawane Adesh et al., 2012). Additionally, high prices as well as the perception of established synthetic sweeteners as being healthy and safe are hard to overcome for natural products to replace synthetics (Neacsu and Madar, 2014). Two primary types of consumers were identified (1. Label-conscious and 2. Flavor-driven). Based on this, natural products would benefit immensely by labels to establish their market share (Parker et al., 2018). Even though the terms “artificial” and “synthetic” are generally connotated negatively, synthetic flavor solutions could still be a targeted and well-designed product for specific purposes (Ulloa, 2018). Besides natural sweeteners and taste-modifiers, synthetically derived sweeteners are found numerously, ranging from Acesulfame-k, to Xylitol (Chattopadhyay et al., 2014). Besides sweeteners, other taste-modifying solutions are commonly known. The most prominent example in the western world is monosodium glutamate associated with the taste of Umami (Bellisle, 1999). Besides known taste-modifying solutions, a new plant-based constituent gains further interest: phyllodulcin, a dihydroisocoumarin that is only present in some *Hydrangea* cultivars being traditionally used as a ceremonial tea in Japan. The history-related overview will be presented in Chapter 2. The general introduction to the *Hydrangea* and secondary plant metabolites will be given in Chapters 1.2 and 1.3 respectively.

A multitude of plants are known to combine their ornamental usage with industrial applications. One representative of these plants is the hortensia – *Hydrangea macrophylla* and its subspecies *H. macrophylla* subsp. *serrata*

1.2 Introduction to the *Hydrangea* species

More than 10 million hortensias are sold in the US alone each year, with around 1,000 species, hybrids and cultivars (Fulcher et al., 2016). Generally, the *Hydrangea* is one genus of the *Hydrangeaceae*. According to Takhtajan, 2009, the taxonomy can be illustrated as follows:

- Phylum Magnoliophyta (Flowering Plants)
- Class Magnoliopsida (Dicotyledons)
- Subclass VII Asteridae
- Superorder Cornanae
- Order 108 Loasales (Hydrangeales)
- Familiy 1 Hydrangeaceae

One of the most prominent examples within the family of Hydrangeaceae is the *Hydrangea macrophylla* (Dirr, 2004). *Hydrangea macrophylla* expresses a decussate phyllotaxis. Plants with a terminal inflorescence have 6–12 nodes, *H. macrophylla* without inflorescence 10–20 nodes. Internode length varies according to the plant's height (Zhou and Hara, 1988). One subspecies of *H. macrophylla* is *H. macrophylla* subsp. *serrata*, also called "tea-hortensia" that will be introduced in more detail in the introductory parts of the following chapters.

1.2.1 Flowering

Hydrangea are well known for their impressive flowers. The color of flowers is mainly dependent on the total amount of Al in the soil and soil pH that affects the accessibility of aluminum (Al). Low soil pH (4.5–5.5) leads to blue flowers due to higher Al accumulation while higher soil pH results in pink flowers (Yeary and Fulcher, 2013). Most *H. macrophylla* cultivars flower in early summer, some throughout the growing period. Some cultivars will not produce blue flowers even given the right framework of soil-pH (4.4–5.5) and total Al (Halcomb and Reed, 2012; Yeary and Fulcher, 2013). General blooming characteristics are cultivar dependent (Weiler, 1980). Anthocyanin content in the sepal is no sufficient indicator for the flowers coloring, because the latter is mainly dependent on abiotic environmental factors, e.g. pH, Al-availability, light exposure (Hariri and Balqis, 2013). Besides photoperiod, air humidity, nutrients, irradiance, and temperature supposedly affect the number and quality of flowers (Eid et al., 2016) while plants with a high amount of leafy biomass and thick stems are more likely to induce blossoms (Struckmeyer, 1950). If not completely sterile, mophead and lacecap hortensias express sterile flowers on the outside and fertile flowers on the inside of the umbel. This was shown to be highly dependent on cultivars as well (Reed, 2005). The sterile decorative flowers were mainly developed to attract pollinators and are highly specific in different *Hydrangea* species and cultivars (Wong Sato and Kato, 2019). Generally, the mainly sterile sepals of *Hydrangea macrophylla* do not attract pollinators like bees the way other flowering plants do (Mach and Potter, 2018).

1.2.2 Shaping and pruning

Regarding growth and flowering different cultivars react differently to environmental factors (e.g. day-length, irradiance etc.). It is advised to choose the cultivar accordingly and adjust the micro-climate to be as fitting as possible (Nordli et al., 2011). In order to keep a *Hydrangea* in shape only little pruning has to be done to allow for a better light-transmission and air circulation between the leaves without depression of growth (Yeary and Fulcher, 2013). *Hydrangea* can be cultivated in containers or in the field. Independent of the cultivation system, the substrate has to be well-drained in order to prevent waterlogging (Owen et al., 2016). For the production of container-grown plants with high biomass accumulation, larger container sizes are advised, as these lead to higher biomass production (Poorter et al., 2012) which was separately investigated for *Hydrangea* as well (Artetxe et al., 1997) as bigger pots also influence the shoot–root ratio and promotes flower initiation. Contrary, the smaller the pot, the more leaves were found in the bud. (Yeh and Chiang, 2001). Not only pot size, but pot composition influences plant performance, as bioplastic pots showed a more uniform root-growth in the pot as well as larger and darker leaves, in comparison to petroleum pots, which might be caused by light transmission through the pot into the growing medium (Huda and Roh, 2014).

1.2.3 Nutrient and water requirements

Symptoms of nutrient deficiencies are not yet clearly defined for different *Hydrangea* cultivars. Still, there are some indicators that are generally recognized. Owen et al. compiled a list of deficiency symptoms for *Hydrangea*. Nitrogen (N) deficiencies lead to chlorotic, or more yellow older leaves that might express dying tips. Younger leaves will express red leaf margins and grow smaller. Young leaves express shortened growth when being exposed to phosphorus (P) deficiencies, while older leaves start to express a purple color. Potassium (K) deficiencies lead to leaf necrosis and slim leaves. A lack of magnesium (Mg), manganese (Mn) or iron (Fe) leads to interveinal chlorosis. Calcium (Ca) deficiency can be recognized by light green to yellow leaves that might be necrotic or disformed. A shortage in sulfur (S) is relatively similar to the one of N. Additionally, the shoot elongation will be reduced which leads to shorter internodes. Profound levels of S deficiencies lead to defoliation (Owen et al., 2016). High amounts of N (210 mg/L and 280 mg/L) led to highest plant biomass, height, number and size of flowers as well as leaf area and chlorophyll content. Still concentration and time of application need further investigation (Bi et al., 2008). *Hydrangea* can be grown on marginal soils, although ideal growing parameters are a moist but not waterlogged soil with high amounts of organic matter. Over-irrigation can cause serious damage to the roots (e.g. root rot) and lead to a shortage in growth (Yeary and Fulcher, 2013). Water restriction leads to plants with less leafy biomass (g/plant and leafs/plant), a lower leaf area and a lower average height (Morel, 2001).

Plant age, leaf area and light intensity influence the water needed by the plant. Using a water supply-model from one year to predict the next years water supply needed showed to be challenging. This is mainly due to variations in plant growth and irradiance and might lead to drastically performed over or under irrigation (O'Meara et al., 2013). Different species react differently on the drying of the soil. Water availability of *Hydrangea macrophylla* as well as soil media have to be considered when trying to find their optimal irrigation regimen (O'Meara et al., 2014).

1.2.4 Sun exposure and cold hardiness

Sunshine is needed for *Hydrangea* cultivation but can lead to wilting if the plants are exposed to direct sunshine. Shading is advised whereas to much shade will decrease the number of flowers produced. High amounts of N will do the same thing but additionally increase the leafy biomass (Yeary and Fulcher, 2013). Still, different cultivars show different levels of sun tolerance (Rinehart et al., 2008) and most favour a cool placement that still allows for enough light to reach the plant (Weiler, 1980).

Hortensias require a cold period (chilling) in order to develop a flower bud. Forcing is possible and leads to an increase in plant height under short day conditions. Under long day photoperiods forced Hortensias grow smaller than unpinched ones (Anderson et al., 2009). A cold period of four weeks is sufficient to produce plants with good quality parameters in flowering and plant height, although some cultivars show better results after three weeks of cooling period (Vidalie, 1986). In USDA climate zone 6 (-23.3°C to -17.8°C) the lack of cold hardiness leads to a damaging of the flower buds which results in a decreased number of flowers. Even in USDA climate zone 7 (-17.8 to -12.2°C) late spring freezes may cause damage in flower buds (Reed, 2000). Generally, *H. macrophylla* can survive in the USDA climate zones 5B to 9A and can grow up to 0.9–1.8 m in height and diameter. *H. macrophylla* requires shade, shows moderate drought tolerance and low soil-salt tolerance. 0.9–1.52 m of plant spacing are required for optimal growth (Gilman, 1999). *Hydrangea* is also referred to being cold hardy in zones 6–9, and growing between 0.9–1.8 m, reaching even up to 3 m while being as wide as high or wider (Halcomb and Reed, 2012). In comparison to *H. paniculata*, *H. macrophylla* is prone to chilling injuries. For both species, temperatures of presumably less than 4°C are needed to start the development of cold hardiness (Pagter et al., 2008).

1.2.5 Pests and diseases

Crop losses can be caused by two separate factors, abiotic and biotic factors. Abiotic impacts on plant performance are irradiation, nutrient supply, temperature, and water supply, while biotic impacts are more diverse but can be summarized into animal pests, pathogens (e.g. fungi, bacteria, viruses), and weeds (Oerke, 2006). Shading-effects on resistance towards leaf-spot diseases were measured under three shading regimens: full shade, full sun and partial shade. *H. macrophylla* subsp. *serrata* was "moderately resistant" or "resistant" to leaf-spot diseases under full shade. The data collected indicates that *H. macrophylla* subsp. *serrata* is slightly less susceptible to leaf-spot diseases than *H. macrophylla* subsp. *marcophylla* (Li et al., 2016). Powdery mildew and bacterial and fungal-caused leaf spots are common diseases in *Hydrangea*, while optimized pruning techniques offer feasible mitigation strategies. Furthermore, several ring spot viruses can occur. If the soil is waterlogged root rot can become a problem. Arthropod pests like aphids, japanese beetles, leaf tiers, rose chafers and spider mites rarely cause significant damage. (Yeary and Fulcher, 2013). Resistant *H. macrophylla* subsp. *serrata* cultivars against powdery mildew are 'Amagi Amacha', 'Diadem', 'Komachi', 'Omacha' and 'Shirofuji'. 'Blue Bird', 'Coerulea', 'Grayswood', and 'Intermedia' are highly susceptible to powdery mildew (Windham et al., 2011).

In their collection "Diseases of Hydrangea" Hagan and Mullen collected the most typical diseases that are recorded for Hydrangeas: anthracnose, armillaria root rot (mushroom root rot), botrytis blight (also known as gray mold), cercospora leaf spot (also reported by Yoshikawa and Yokoyama, 1992 and Nakashima et al., 2010), Phytophthora root rot and powdery mildew (Hagan and Mullen, 2001). A similar list was published by Baysal-Gurel, Kabir and Blalock in 2016: Anthracnose, Alternaria Leaf Spot, Botrytis Blight, Cercospora Leaf Spot, Phyllosticta Leaf Spot, Powdery mildew and Rust. In addition, the authors listed bacterial and viral diseases. In the first category bacterial leaf spot and bacterial wilt were classified as important, but mainly infect *H. quercifolia*, *H. macrophylla* and *H. arboreacens*. In the latter category there are 15 different viruses listed: Alfalfa mosaic virus, Arabis mosaic virus, Cherry leaf roll virus, Cucumber mosaic virus, Hydrangea mosaic virus, Hydrangea latent virus, Hydrangea ringspot, Hydrangea chlorotic mottle virus, Impatiens necrotic spot virus, Tobacco necrosis virus, Tobacco rattle virus, Tobacco ringspot virus, Tomato blackring virus, Tomato ringspot virus and Tomato spotted wilt virus (Baysal-Gurel et al., 2016). Hydrangea ringspot virus, alfalfa mosaic virus and tobacco ringspot virus occur on different *Hydrangea* species and mainly infect certain cultivars (Chiko and Godkin, 1986). Significant pests during *Hydrangea* production are spider mites (*Tetranychus kanzawai*), as well as garden thrips (*Frankliniella intonsa*), and aphids

(*Aphis gossypii*) that cause enough damage to be eligible for plant protection techniques (Ma et al., 2015).

Besides pests and diseases known for a long time, new ones are constantly found. Thus, *Ramularia hydrangeicola*, causing leaf spots on *H. serrata* f. *acuminata* was first described in 2016 (Park and Shin, 2016). Recently, *Telimena hydrangea*, a tar spot fungus was described (Mardones et al., 2018) as well as the the population dynamics of *Tetranychus kanzawai* (kanzawa spider mite) by Gotoh and Gomi (Gotoh and Gomi, 2000).

1.2.6 Propagation

There are mainly three ways of propagation for *Hydrangea* species, each presenting its own advantages and disadvantages: Propagation by seeds, stem cuttings, and micropropagation. Propagation by seeds is mainly used for breeding and selection for specific plant characteristics. The handling of the very small seeds can be rather challenging. Generally, no pre-treatments are needed for *Hydrangea* species to germinate within 10–30 days. Germination speed can be increased by stratification for 30–90 days. Seeding in a greenhouse under controlled humidity is advised (Owen et al., 2016). Besides other parameters (e.g. soil parameters), the date of cutting is of mayor importance when propagating *Hydrangea* via cuttings. Softwood cuttings are a lot more likely to root (80% rooting) and produce longer roots (mean 0.9 cm) compared to hardwood cuttings (20% rooting; 0.6 cm mean root length) (Park and Kim, 1993). Cuttings from end of June grew significantly higher, produced more side branches than cuttings from mid of August, and generally reached a higher quality ranking than cuttings from August (Midcap, 2005). Soft wood cuttings are ideally collected earlier in the season in order to achieve faster growing and robust plants, but might grow asymmetrically in comparison to micropropagated plants. Micropropagation allows to produce a high number of symmetrical plants in a short time, that show shorter internodes and a full canopy with many branches and a balanced root–shoot ratio. Furthermore, micropropagated plants show higher survival rates when being transplanted which might be due to more developed root systems and more axilliary buds. Downsides of micropropagated plantlets for *Hydrangea* production are that acclimatization can be problematic and the plants are more fragile than plants from seeds or stem cuttings. Additionally, they require more shade and irrigation when being acclimated (Owen et al., 2016).

Hydrangea is mainly known as an ornamental plant and was studied in this regard intensively. Some cultivars contain the dihydroisocoumarin phyllodulcin that enables the plant to be categorized as a taste-modifier within the renewable resources as well.

1.3 Introduction to secondary metabolites and dihydroisocoumarins

The tea-hortensia possesses a unique feature in the synthesis of phyllodulcin, a secondary metabolite which makes it so interesting as an industrial crop. Secondary metabolites in plants are frequently affected by biotic and abiotic stressors, e.g. habitat, water and nutrient availability, UV radiation or leaf damage (Gobbo-Neto and Lopes, 2007) and play a significant role in defense mechanisms, while being environmentally as well as genetically influenced (Ramakrishna and Ravishankar, 2011; Lattanzio, 2013). Light is an important factor in addressing secondary metabolites, as not only Photosynthetically Active Radiation (PAR, 400–700 nm) contributes to their composition, but Physiologically Active Radiation (300–800 nm) as well (Thoma et al., 2020). Medicinal plants are rich in secondary metabolites which leads to their usage for pharmacological purposes (Savithramma et al., 2011). Furthermore, the beneficial contribution to health improvement by plant material like cereals, vegetables or fruits is heavily attributed to phenolic compounds (Cheynier, 2012; Khoddami et al., 2013).

Phenolic compounds are an overview term for aromatic substances with an aromatic ring and at least one hydroxyl substituent. Phenolic compounds usually occur in glycosides with the largest group of phenolic compounds being flavonoids (Harborne, 1973). Phenolic compounds are omnipresent in plants (Balasundram et al., 2006) and can be grouped into three categories, simple phenols and phenolic acids, derivatives of hydroxycinnamic acid, and flavonoids (Ho, 1992). Synthesis of phenols is driven by the shikimate/phenylpropanoid pathway which branches off to the flavonoid branch pathway or the "polyketide" acetate/malonate pathway (Lattanzio, 2013). One compound build via the shikimate-phosphate pathway is coumarin (Yang et al., 2018). Isomers of coumarin are called isocoumarins and are grouped according to their oxygen or carbon substituents at the lactonic or benzene rings. The saturated analogue of isocoumarin is the 3,4-dihydroisocoumarin (DHC): 3,4-dihydro-1*H*-2-benzo[c]pyran-1-one (Saeed, 2016). DHCs are present in bacteria, fungi, marine organisms, and higher plants (Shabir et al., 2021). One of these 3,4-dihydroisocoumarins is hydrangenol (HG), which is the precursor of phyllodulcin (PD) (Yagi et al., 1977), the main contributor to the taste of the traditional tea from *H. macrophylla* subsp. *serrata* (Yasuda et al., 2004). The yield of hydrangenol and phyllodulcin can be optimized via targeted post-harvest treatment. Destructive treatment of leaves can increase the turnover from phyllodulcin glycosides into their aglycones (Jung et al., 2016). Additionally, post-harvest treatments (fermentation or freezing) of leaf material can counteract negative effects of high drying temperatures (Moll et al., 2021a). Phyllodulcin ($C_{16}H_{14}O_5$) holds a lot of synonyms with the most commonly known being (R)-8-Hydroxy-3-(3-hydroxy-4-methoxyphenyl)isochroman-1-one. While the general pathway of synthesis is known, detailed information on enzymes and intermediates remains unclear and further investigations are needed.

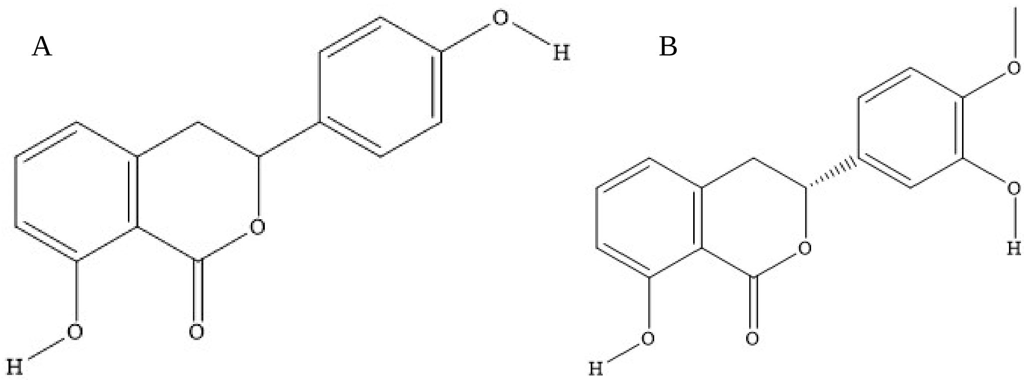

Figure 1. Structural formula of hydrangenol (A) and phyllodulcin (B) according to PubChem, visualized using ChemDraw Prime (PerkinElmer Informatics)

As more than 400 isocoumarins are known today (Yin et al., 2010; Saeed, 2016) and multiple review articles dealt with their pharmacological as well as taste or flavor properties (Barry, 1964; Hill, 1986; Pal et al., 2011; Saddiqa et al., 2017). Phenolic compounds in general are a major contributor to sensory and nutritional quality of foods (Ho, 1992) and as part of this, the same holds true for isocoumarins and dihydroisocoumarins like phyllodulcin (Kim et al., 2016b). Still, besides the commonly known pharmacological properties, innovative ways of usage are an extensive field of research as well (Vaishnav and Demain, 2011). While absorption, metabolism and excretion of phenolic compounds was researched intensively, other parameters like impact of food matrix and the combination with other foods as well as nutrients is still subject to further research (Velderrain-Rodríguez et al., 2014).

Spectral methods are well usable for identification and quantification of such structures and is possible in the ultra violet (UV) as well as infrared (IR) region (Harborne, 1973). Near infrared (NIR) spectroscopy seems to be a promising tool for the determination of phenolic compounds (Pissard et al., 2018) by combining a reference method (HPLC, UV-VIS, chemical) with the infrared method (Cozzolino, 2015). This quality control in regard of phenolics can be performed in raw materials and finished products in a wide range of cosmetics, food industry, and pharmaceuticals (Acosta-Estrada et al., 2014).

Secondary metabolites fulfill multiple purposes in plants and can be exploited for human use as well. Detection of these metabolites can be performed via laboratory analytics and the arising technologies of hyperspectral analysis.

1.4 Non-invasive plant phenotyping

The possibilities of non-invasive phenotyping form one pillar in the methods used in this thesis. From measuring plant biomass to modeling of hydrangenol and phyllodulcin contents, a multitude of techniques were used. In this part, the fundamentals of non-invasive phenotyping are presented to lay the foundation for later chapters with their respective practical hands-on measurements in tea-hortensias.

1.4.1 Introduction and definitions

The terminology of "phenotyping" is commonly used for the description of the plant phenotype. The quantitative description of plant properties (anatomy, ontogeny, physiology, and biochemistry) is a key feature of plant phenotyping (Walter et al., 2015). For this work, the definition of Fiorani and Schurr will be used as it defines phenotyping as "the set of methodologies and protocols used to measure plant growth, architecture, and composition with a certain accuracy and precision at different scales of organization, from organs to canopies" with a special focus on non-invasive phenotyping (Fiorani and Schurr, 2013). Examples for commonly used destructive measurements are root morphology investigations or yield-related parameters (Chiluwal et al., 2018). Non-invasive phenotyping aims at combining qualitative and quantitative estimations of plant parameters (Mahlein et al., 2019). Generally, two types of non-invasive phenotyping are possible: non-imaging and imaging techniques.

The first group relies on the interaction of light with the plants structures to be measured. Light can traverse the leaf (transmittance), be absorbed by chemicals in the leaf (absorbance) or reflected by structures of the leaf surface (reflectance) (Mahlein et al., 2018). The light used for such applications differs in wavelength depending on the structures that are to be measured, ranging from UV, (100-380 nm) (Brugger et al., 2019), to visible (400–780 nm) and infrared (800–25,000 nm) light (Cozzolino, 2015; Asgari et al., 2020). Physics of such applications revolve around the Kubelka-Munk-Theory while many physical theorems play a role in reflectance spectroscopy (Simmons, 1975; Clark and Roush, 1984) which will not be discussed in this context. Reflectance spectroscopy is not limited to plants or material sciences but is also used in human medicine (Wallace et al., 2009).

The second group (imaging phenotyping) is based on a multitude of different techniques that partially overlap with non-imaging techniques as well. A comprehensive overview of imaging techniques was given by Li et al. (2014). Imaging techniques can be used in two-dimensional (2D) and three-dimensional (3D) formats. 2D uses threshold based, edge based, region based, clustering based, or deep learning methods. 3D images on the other hand require a different processing, starting with image acquisition and image processing before image analysis finalizes this procedure (Li et al., 2020). Imaging techniques can be classified in visible light imaging, fluorescence imaging, thermal imaging, near infrared (NIR) imaging, hyperspectral imaging, 3D imaging, and laser imaging. Li et al. incorporate magnetic resonance imagers (MRI), positron emission detectors (PET) and X-ray computed tomography (CT) in their review as well (Li et al., 2014). This list can be extended for the different sensors used for such applications as compilated by Qiu et al.: stereo vision systems, lidar/laser sensors, range cameras, spectral sensors and cameras, thermography, fluorescence and ultrasonic sensors as well as thermometers (Qiu et al., 2018). In short, plant phenotyping aims at "uncovering the hyperspectral language of plants" (Wahabzada et al., 2016).

1.4.3 Areas of utility

Non-invasive and rapid phenotyping provides a profound tool for a lot of applications. A particularly interesting one is the forecasting of yield and ideal harvest time (Koirala et al., 2019; Anderson et al., 2021) as well as food or product quality (Cen and He, 2007; Cozzolino, 2015). Yield optimization is the most intensively researched aspect in plant optimization via phenotyping (Rascher et al., 2011). Additionally, stress and stress tolerance can be quantified (Chiluwal et al., 2018; Fujita et al., 2018). Non-invasive phenotyping allows for the simultaneous quantification of parameters to investigate interactions, e.g. stress and photosynthesis (Jansen et al., 2009) and presents a promising way in the field of phytopathology (Mahlein, 2016; Mahlein et al., 2018) as well as vitality monitoring during plant production (Ruett et al., 2022). Investigations on photosynthesis allow for a precise irrigation management and therefore an increased water use efficiency (Haworth et al., 2018). Phenotyping is not limited to photosynthesis or yield prediction, but the right equipment also allows for plant height, leaf area index (LAI), chlorophyll, water stress, and biomass assessment (Qiu et al., 2018). Based on reflectance spectra, qualitative and quantitative information can be obtained via simple and effective algorithms resulting in vegetation indices, or "VIs" in short (Xue and Su, 2017). While the computation is simple, the interpretation of such indices requires knowledge of input variables as well as the basics of index calculation. If used correctly, VIs reveal information regarding biomass, water status, different stresses and health (Jackson and Huete, 1991). The reflectance spectroscopy not only is performed by hand-held devices but can be performed via remote sensing as well (Huete, 2012). By choosing the suited sensor for the respective application, on-farm measurements can be performed throughout the day with close to none limitations (Souza et al., 2021).

The linkage of plant phenotype and genotype is a particularly interesting development within plant phenotyping (Mishra et al., 2016). Monitoring known traits and discovering new ones during plant selection processes is a promising way in plant breeding (Großkinsky et al., 2015a) and presents the possibility to close the gap between genotype and phenotype (Großkinsky et al., 2015b). Genetic loci for salinity tolerance in rice (*Oryza sativa* L.) were identified using plant phenotyping (Al-Tamimi et al., 2016) while growth related parameters in maize (*Zea mays* L.) were also revealed via non-invasive phenotyping (Muraya et al., 2017). Still, non-invasive measurements have to be validated and re-investigated in a lab to ensure quality of measurements. A prominent example of this are photosynthetically active pigments, e.g. chlorophylls, carotenoids and tocopherols (Junker and Ensminger, 2016).

The incorporation of Unmanned Aerial Vehicles (UAVs) solves a fundamental problem in plant phenotyping: the time consumption of data collection. Consequently a new problem arises by the amount of data: the data processing (Kefauver et al., 2017). Deep learning techniques enable extraction of relevant information from complex and multidimensional datasets (Koirala et al., 2019) by being applicable for identification, classification, quantification, and prediction (Singh et al., 2018). The combination of quantitative and qualitative traits can only be successful by linking sensor data, machine learning (or other statistical analysis) with the specific knowledge on plant traits (Mahlein et al., 2019). This linkage could lead to a cycle from phenotyping, computation, analysis to breeding selection to start the next cycle consecutively (Shakoor et al., 2017). Additionally, networks and collaborations of stakeholders in plant phenotyping are needed to provide the highest amount of benefit by maximizing the value of generated data (Rosenqvist et al., 2019; Morisse et al., 2022). The investigations of samples in an agricultural context are not limited to plants and products but extend to soil properties as well. The latter can be quantified using NIR reflectance spectroscopy in combination with adequate statistical analysis (Chang et al., 2001).

As ever new phenotypic markers and phenotyping technologies are developed to understand plants better, the following two examples provide an outside-the-box view on phenotyping: the emission of volatile organic compounds from plants (Niederbacher et al., 2015) as well as the emitting of sound in the range of 20–100 kHz as shown for drought stress (Khait et al., 2018) under stress. Besides the development of new techniques, new and purpose-adapted sensors are a key component for the future of plant phenotyping (Pieruschka and Schurr, 2019). Setups based of commercially available components can be assembled into a reliable "phenotype collection system" for grow chambers for around $250 (Tsaftaris and Noutsos, 2009).

Plant phenotyping provides a multipurpose toolbox for a wide range of applications that enables researchers to answer a wide range of scientific questions.

1.5 Importance and research objectives

The main objective of this thesis is to provide insight into basic cultivation and quality assessment for tea-hortensias, *H. macrophylla* subsp. *serrata*. Based on the literature review in Chapter 1, the following chapters go into more detail on the specifics regarding the aspects presented in the respective chapters. The investigations are based on multiple experiments presented in those chapters.

First, the effect of shading on three different tea-hortensia cultivars was investigated to evaluate the impact of shading on biomass accumulation as well as HG, PD, and DHC contents. For the estimation of plant performance, hyperspectral data was used as basis for calculating Vegetation Indices. Those VIs allow for a fast and simple estimation of plant health and performance (Chapter 2). Additionally, this chapter established basic knowledge on hydrangenol and phyllodulcin contents for the cultivars used throughout this thesis.

Hypothesis I and II:

Tea-hortensia cultivars require shading for optimal productivity

Cultivars react differently to increased light exposure

As a special focus of this study lies on the dihydroisocoumarin phyllodulcin, the dynamics of hydrangenol and phyllodulcin are investigated in chapter 3. External effects (abiotic and biotic stress, bio-rationals, etc.) seem to play a minor role as the genetic preposition of PD synthesis seems predetermined. In the chain of synthesis, the step from hydrangenol to phyllodulcin is a key turning point, as most of *H. macrophylla* accumulate HG, but only tea-hortensia synthesizes PD from it. A quantitative estimation of this conversion is needed and could be highly beneficial for plant breeding, as not only total PD content but also the relative conversion from HG to PD is a beneficial trait for future crosses with tea-hortensias. This trait can be estimated by calculating an index of tea-hortensia PD-synthesis, the chemometric Normalized Difference Phyllodulcin Index – cNDPI (chaper 3). After understanding the HG and PD contents in tea-hortensia cultivars under different shading levels, this chapter adds the quantification of dihydroisocoumarin conversion.

Hypothesis III and IV:

Tea-hortensia cultivars produce HG and PD at different ratios that can be used as chemometric marker

The proposed index "cNDPI" is a feasible and reliable tool for plant selection during breeding

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Besides as basis for the calculation of VIs, hyperspectral data can be used to estimate HG and PD contents in the leaves via statistical modeling. This modeling enables a farmer to predict optimal harvest dates, as no invasive measurements are to be taken and long-lasting laboratory analysis are not needed. The modeling of hyperspectral data is presented in Chapter 4 to investigate the possibilities and limitations of such models as a decision support for harvesting as well as fast estimation of treatment-reaction interaction for research purposes. Additionally, the impact of measurement conditions as well as the possibilities of sole cultivar differentiation are investigated (Chapter 4).

Hypothesis V and VI:

Measurement conditions influence model accuracy

VIS-NIR models are suitable to predict HG and PD contents

The three research objectives with their six hypotheses lead to the first overview on the possibilities of quality assessment for tea-hortensias and the impact of cultivation techniques. The contents of this overview can be seen as first indicators for farmers and producers that want to enter the market of dihydroisocoumarin or especially phyllodulcin production and plant breeders for the production of highly productive tea-hortensias.

Chapter 2 – Dihydroisocoumarin Content and Phenotyping of *Hydrangea macrophylla* subsp. *serrata* under different shading regimes

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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 (Yasuda et al., 2004). The originally wildly growing plant is nowadays cultivated in smaller gardens (Ujihara et al., 1995). Various common names have been proposed for *H. macrophylla* subsp. *serrata*, such as San-soogook, Mountain Hydrangea, Tea of Heaven (Shin et al., 2019) and Amacha (Kawamura et al., 2002), 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 (McClintock, 1957). 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., van Gelderen and van Gelderen, 2004; Ohba and Akiyama, 2013, 2014)). 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 (Reed and Rinehart, 2006). Still, *H. serrata* is used frequently in scientific writings (e.g., Matsuno et al., 2008; Ryu et al., 2010; Yin et al., 2010; Meeboon and Takamatsu, 2015; Suyama et al., 2015; Owen et al., 2016) and for convenience (van Gelderen and van Gelderen, 2004; Reed and Rinehart, 2006).

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 (Yasuda et al., 2004). 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 (Ujihara et al., 1995). 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 (Kindl, 1971). Besides PD, hydrangenol (HG), a precursor of PD (Yagi et al., 1977) that is also present in other *Hydrangea* species (e.g., *H. macrophylla* 'Engel's White') (Asen et al., 1960), 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 (Loconsole and Santamaria, 2021). Abiotic stress caused by high light intensity can be managed through shading (Ferrante and Mariani, 2018). In commercial *Hydrangea* production, shading is essential to prevent wilting, but higher levels of shading will decrease flowering (Yeary and Fulcher, 2013) or could lead to flower greening (Kesumawati et al., 2009). 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 (Castro et al., 2006). 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 (Surjadinata et al., 2017). 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 (Rouphael et al., 2018). 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 (Castellano et al., 2008). The reduction of UV radiation limits plant stress and promotes healthy and vital plants (Müller-Xing et al., 2014). 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 (Stigter, 1984). During the production of ornamental plants, the influence of light scattering, mainly for shaping and flowering control, is used (Nissim-Levi et al., 2008).

Plant phenotyping provides a tool for health assessment in plants, for example, by monitoring leaf traits (Scharr et al., 2015), photosynthesis (Jansen et al., 2009) or stress (Masuka et al., 2012). Vegetation indices provide information about a multitude of different plant parameters. These indices are calculated using different mathematical operations on reflectance spectra (Bannari et al., 1995). 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 (Gamon et al., 1995). 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 (Hatfield and Prueger, 2010). The Photochemical Reflectance Index (PRI) is mainly used as a proxy for light-use efficiency (Garbulsky et al., 2011). Leaf pigment-related indices can be grouped according to Roberts et al. to distinguish chlorophyll, carotenoids and anthocyanin (Roberts et al., 2011). 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 (Horler et al., 1983; Boochs et al., 1990). Pigment changes can also be measured by leaf color (Greer, 2005), which can be described by hue angle (h°). Hue angle illustrates a color gradient from 0 or 360° hue (red) to 180° hue (green) (McGuire, 1992).

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', 'Oa-macha' 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. Figure1 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 X12 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 (McGuire, 1992) using MS Excel 2019.

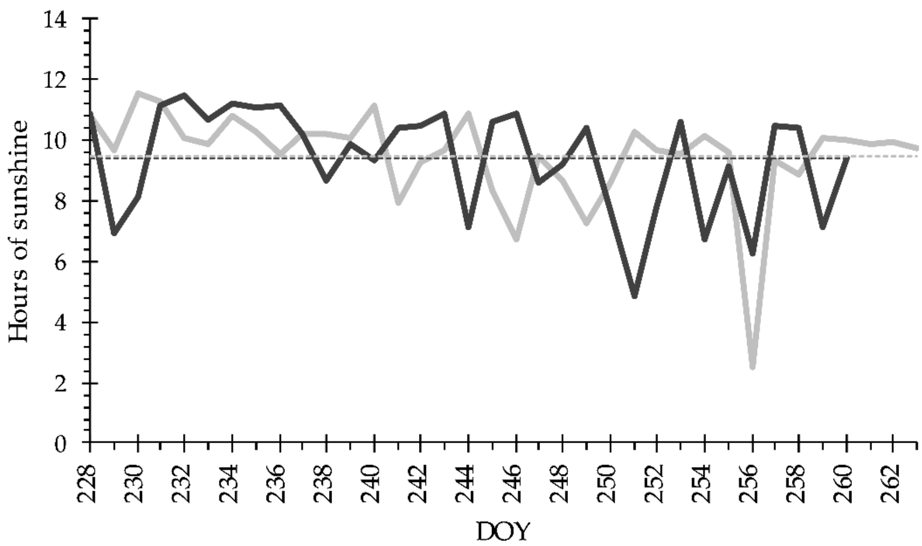


Figure 1. Hours of sunshine (hour of sunshine >120 W m^{-2}) in 2018 (light gray) and 2019 (dark gray) over the course of the experiment (in DOY). Dotted lines represent mean hours of sunshine for the years. Complete data are available via the Supplementary Materials (Table S1).

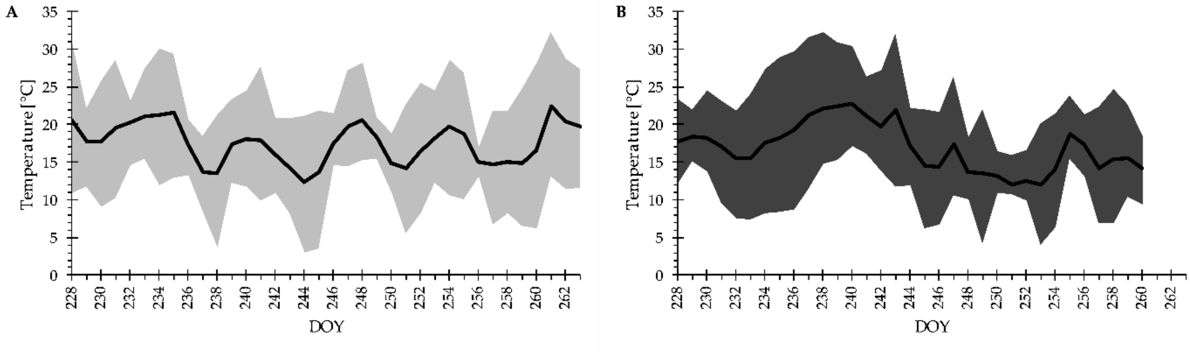
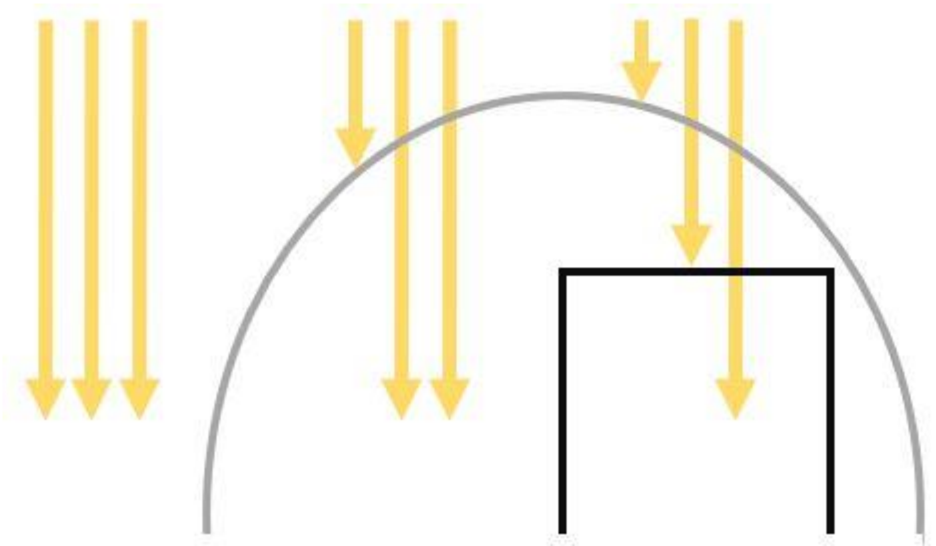


Figure 2. Mean daily temperature (°C) in 2018 (A) and 2019 (B). Shaded area indicates minimum and maximum temperatures. Complete data are available via the Supplementary Materials (Table S1).



	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^{-2s-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	$NDVI = \frac{(R_{NIR} - R_{RED})}{(R_{NIR} + R_{RED})}$	(Rouse et al., 1974)
Chlorophyll	Modified Chlorophyll Absorption in Reflectance Index 1	$MCARI\ 1 = 1.2 * (2.5 * (R_{790} - R_{670}) - 1.3 * (R_{790} - R_{550}))$	(Haboudane et al., 2004)
Chlorophyll	Greenness Index	$G = \frac{R_{554}}{R_{677}}$	(Zarco-Tejada et al., 2005)
Anthocyanins	Anthocyanin Reflectance Index 1	$ARI\ 1 = \frac{1}{R_{550}} - \frac{1}{R_{700}}$	(Gitelson et al., 2001)
Carotinoids	Carotinoid Reflectance Index 1	$CRI\ 1 = \frac{1}{R_{510}} - \frac{1}{R_{550}}$	(Gitelson et al., 2002)
Light use	Photochemical Reflectance Index	$PRI = \frac{(R_{531} - R_{570})}{(R_{531} + R_{570})}$	(Gamon et al., 1992)
Stress	Plant Senescence Reflectance Index	$PSRI = \frac{R_{678} - R_{500}}{R_{750}}$	(Merzlyak et al., 1999)
Stress	Red-Edge Inflection Point 1	$REIP\ 1 = 700 + 40 * (\frac{\left(\frac{R_{670} + R_{780}}{2}\right) - R_{700}}{R_{740} - R_{700}})$	(Clevers et al., 2002)

[1] Indices are grouped according to Roberts et al., 2011). [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 x 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 (Ujihara et al., 1995). 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 (Ujihara et al., 1995). A multi-variate 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.

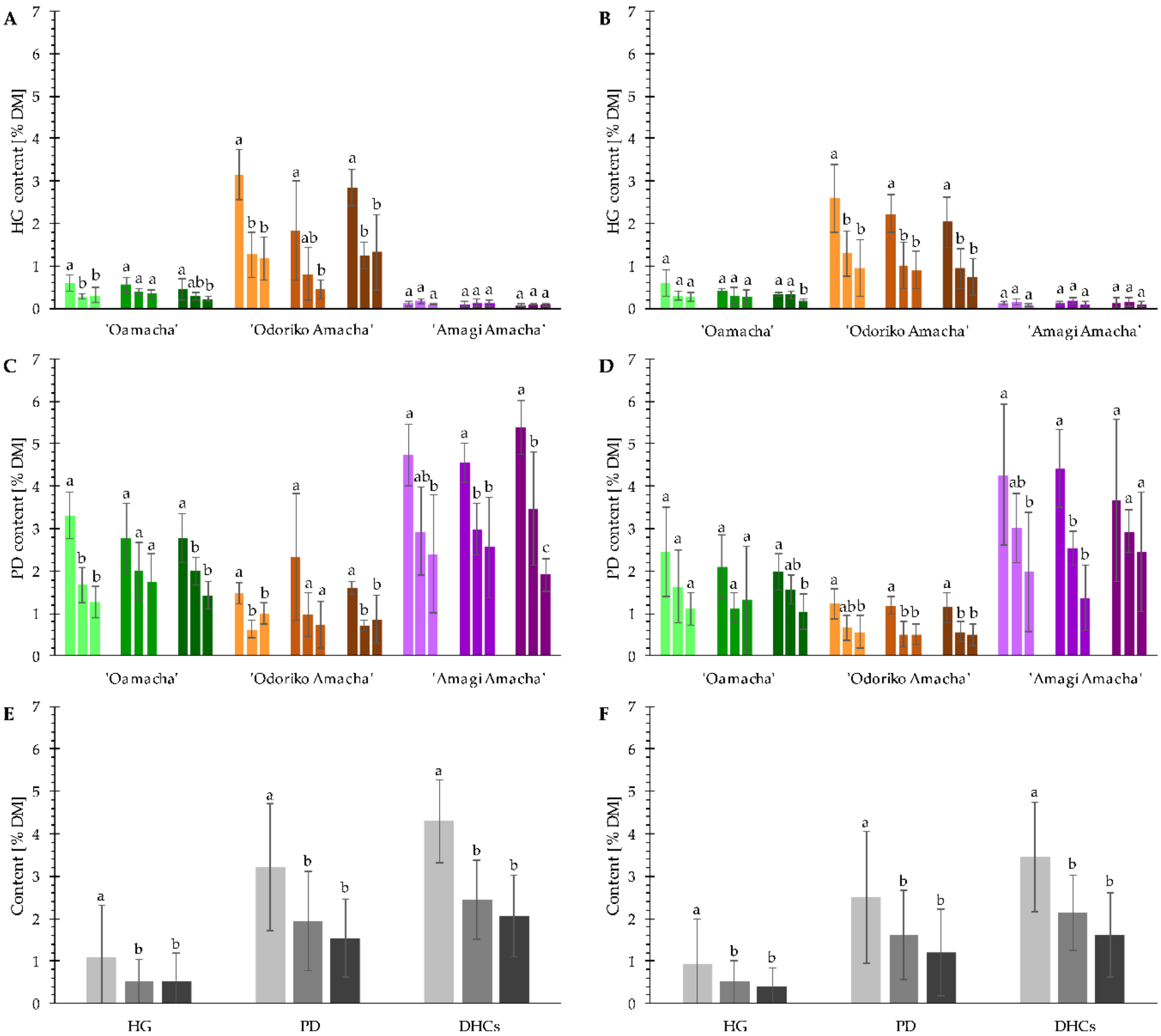


Figure 4. Content (%) of (A,B) hydrangenol (HG) and (C,D) phyllodulcin (PD) for three H. macrophylla subsp. serrata cultivars (green: ‘Oamacha’, orange: ‘Odoriko Amacha’, purple: ‘Amagi Amacha’) and (E,F) for HG, PD and DHC content pooled over all cultivars depending on three shading scenarios in leaf 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 ($n = 5$, $\alpha = 0.05$) 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* (Dietrich and Valio, 1973). Besides that, HG seems to have nearly no beneficial biological activity for the plant (Asen et al., 1960).

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 (Yagi et al., 1977), 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 4 E,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 Figure5, 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 ± 0.58%), with significantly higher HG in 2019 (3.16 ± 0.44%) compared to 2018 (2.61 ± 0.73%) when pooled over all treatments. ‘Amagi Amacha’ seems to convert HG into PD at a very high rate, as only 0.1 ± 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 (Nesi et al., 2013). 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.

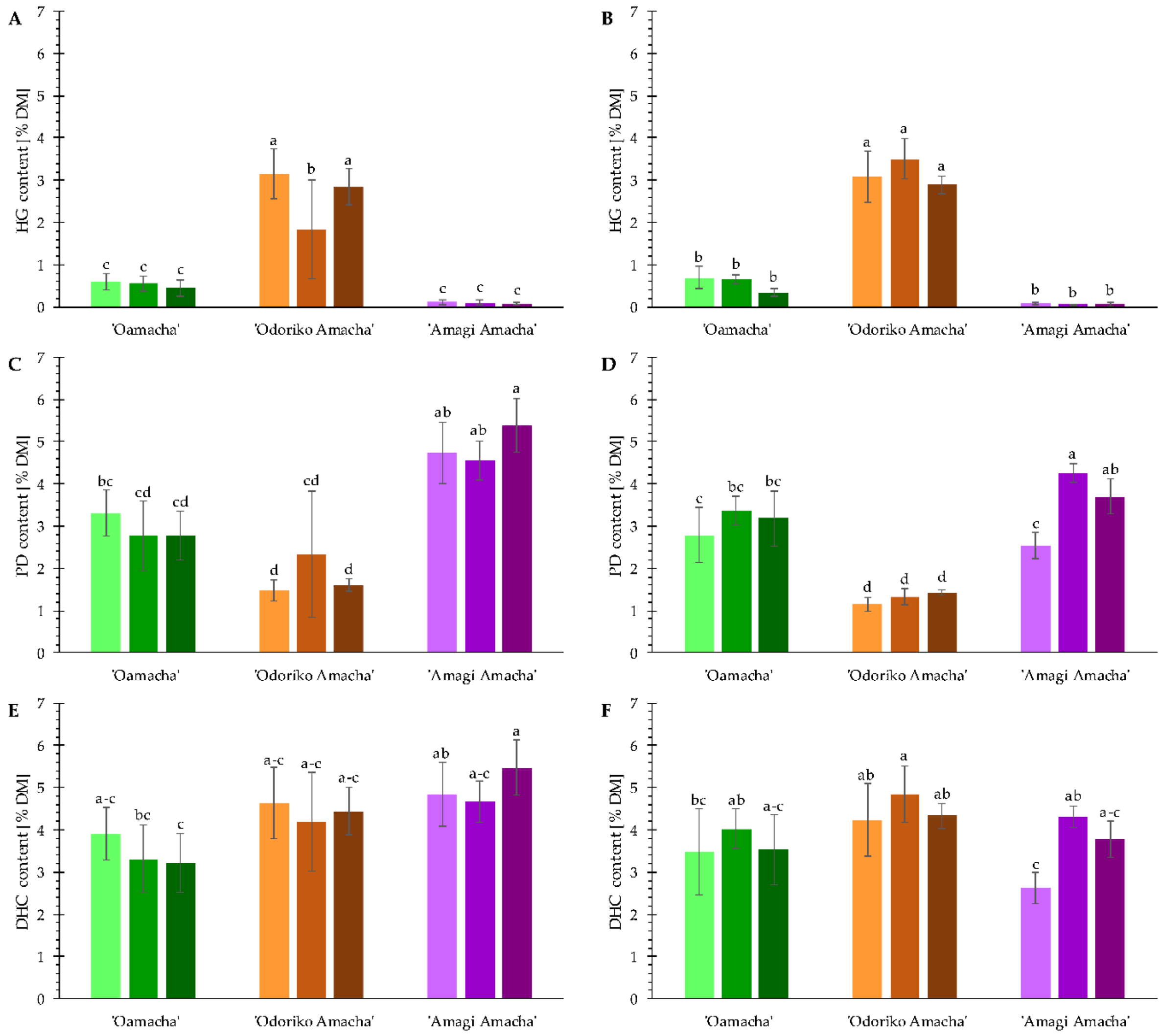


Figure 5. Content (%) of **A**, **B** Hydrangenol (HG), **C**, **D** Phyllodulcin (PD) and **E**, **F** Dihydroisocoumarin (DHC) in leaf dry matter of tea-hortensia cultivars (green: ‘Oamacha’, orange: ‘Odoriko Amacha’, purple: ‘Amagi Amacha’) in two different years (**A**, **C**, **E**: 2018 and **B**, **D**, **F**: 2019) at the end of two growing periods under three different light conditions (light coloring: no shade, darker coloring: partial shade, dark coloring: fullcshade). Significant differences within a year and constituent calculated by ANOVA and Tukey-HSD (n=5, α=0.05) are indicated by letters (a-d).

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 (Cochran and Fulcher, 2013). Combinations of pinching or pruning and dikegulac sodium influence not only branch numbers but also leaf area (Sun et al., 2015). 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 (Hester et al., 2013).

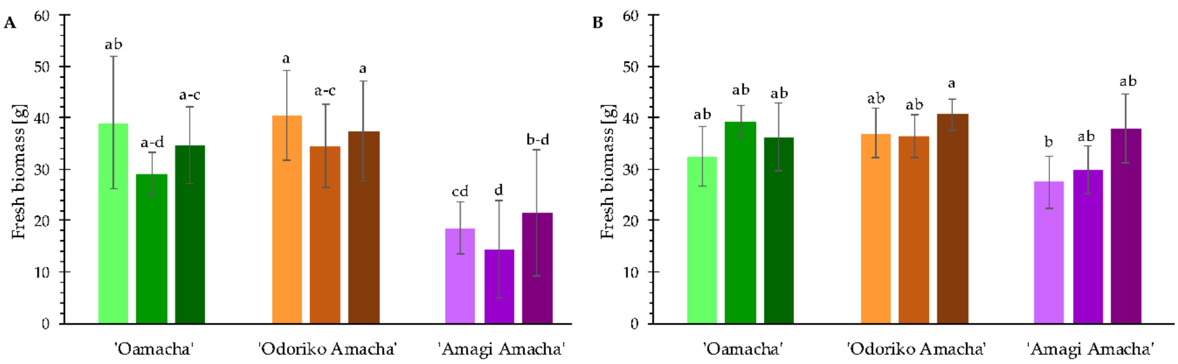


Figure 6. Total fresh biomass (g) of *H. macrophylla* subsp. *serrata* (green: ‘Oamacha’, orange: ‘Odoriko Amacha’, purple: ‘Amagi Amacha’) in two different years ((**A**): 2018 and (**B**): 2019) at the end of two growing periods under three different light conditions (bright coloring: no shade, light coloring: partial shade, dark coloring: shade). Significant differences within a year calculated by ANOVA and Tukey HSD ($n = 5$, $\alpha = 0.05$) are indicated by letters (a–d). Mean ± 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* (Yagi et al., 1977). 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 (Yeary and Fulcher, 2013). Sun tolerance of *Hydrangea macrophylla* has been shown to be cultivar dependent (Condon, 2017). 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 8 A2, B2, C2) at the last day in 2018. This shift was also observed for 'Oamacha' and 'Amagi Amacha' but to a lesser extent.

1	a	a	a	a	a	a	a	a	a	1	a	a	a	a	a	a	b	a	a
2	a	a	a	a	a	a	b	b	b	2	a	b	b	a	b	a	a	a	a
3	a	a	a	a	a	a	a	a	a	3	b	b	a	b	ab	a	a	a	a
4	a	a	a	a	a	a	a	a	a	4	a	b	a	a	a	a	a	ab	b
5	*	*	*	*	*	*	*	*	*	5	*	*	a	a	a	a	a	*	a

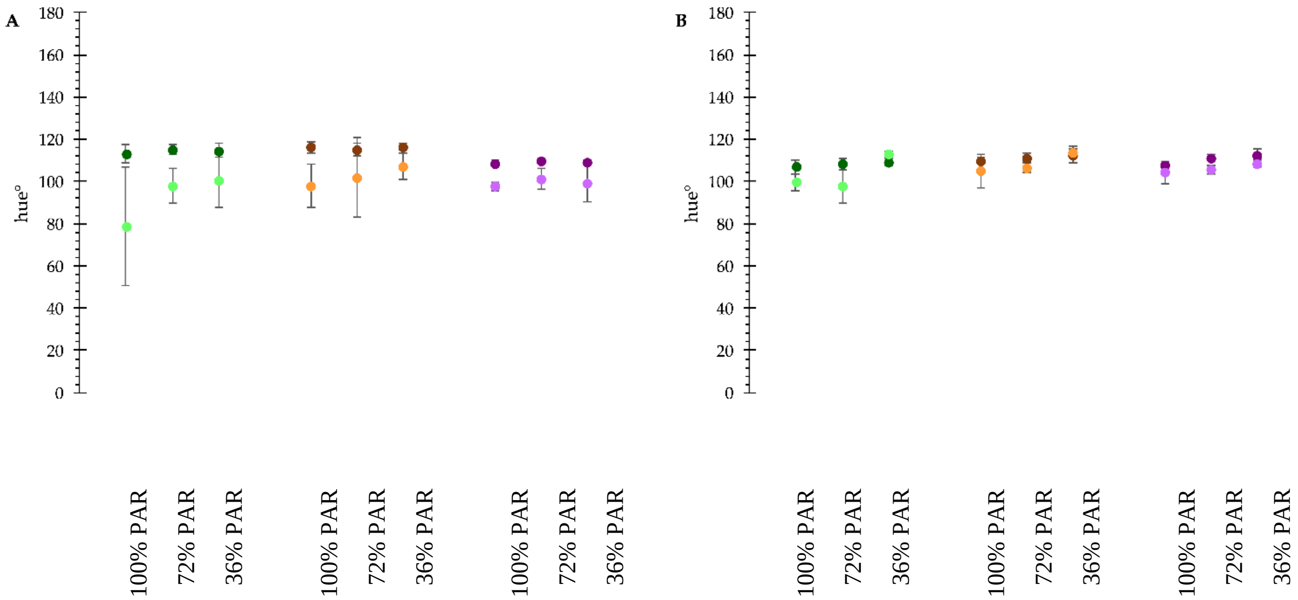


Figure 7. Hue angle of three different *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') at first (dark coloring) and last (light coloring) day of measurements in two cultivation periods ((A): 2018; (B): 2019). Shading regimes are indicated 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 ($n = 5$, $\alpha = 0.05$). 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 9 A,B). Anthocyanins can function as a reducing factor for photoinhibition and leaf damage (Steyn et al., 2002). Therefore, the photoinduction of anthocyanins via UV, Vis and far-red wavelengths has been described (Chalker-Scott, 1999). In addition to the photoinhibition-related effects, nutrient deficiencies in phosphorus (P) or nitrogen (N) are indicated by purpling due to anthocyanin accumulation (Steyn et al., 2002).

The CRI 1 as an indicator for carotenoids only showed significant differences between no shade and (partial) shade in ‘Oamacha’ (Figure 9 C,D; Table S5). It was shown that carotenoids can be an indicator for high irradiance adaptation of leaves (Sarijeva et al., 2007). 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 9 G,H) and the Greenness Index, G (Figure 9 E,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 (Strid and Porra, 1992; Marwood and Greenberg, 1996; Mahdavian et al., 2008).

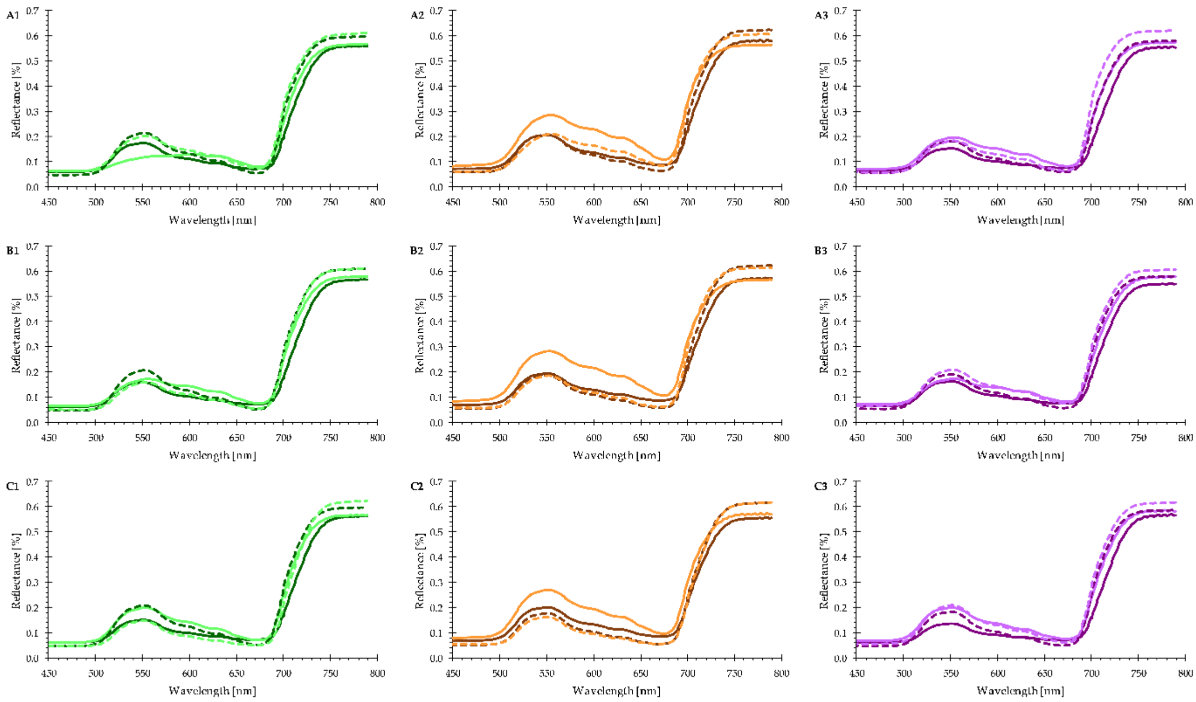


Figure 8. Reflectance spectra of three different *H. macrophylla* subsp. *serrata* cultivars (1: 'Oama-cha', 2: 'Odoriko Amacha', 3: 'Amagi Amacha') under three different shading regimes ((A): no shade, (B): partial shade, (C): full shade). Full lines represent 2018 and dotted lines 2019, with dark coloring 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 'Oama-cha' (Figure10 A,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 (Thenot et al., 2002). 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 (Angert et al., 2005). Generally, the PRI is a reliable proxy for photosynthetic efficiency (Garbulsky et al., 2011). In conclusion, higher amounts of shading seem to increase photosyn-thetic 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.

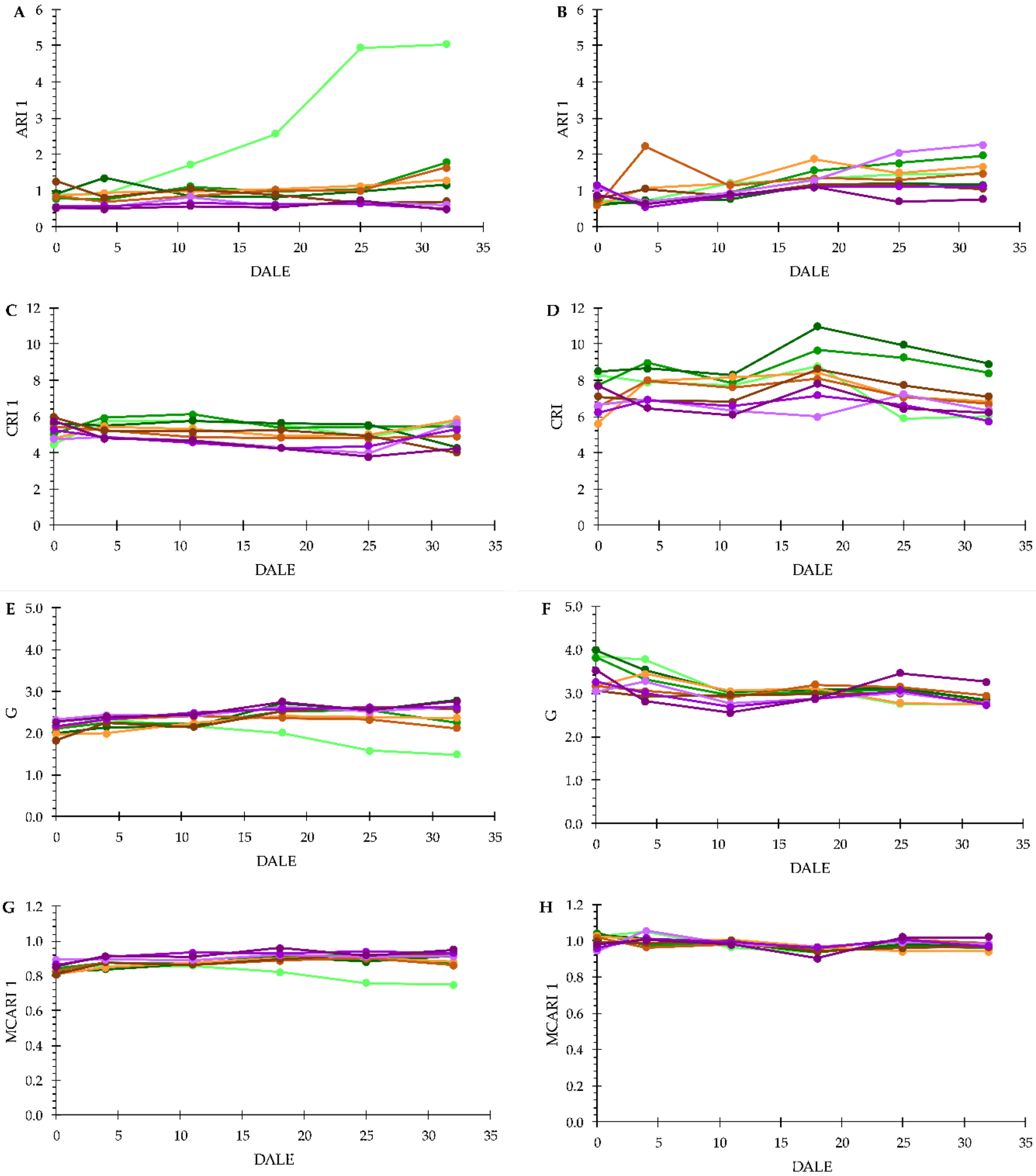


Figure 9. Pigment-based vegetation indices for *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') under different shading conditions (dark coloring: full shade, medium coloring: partial shade, light coloring: no shade) in 2018 (A,C,E,G) and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

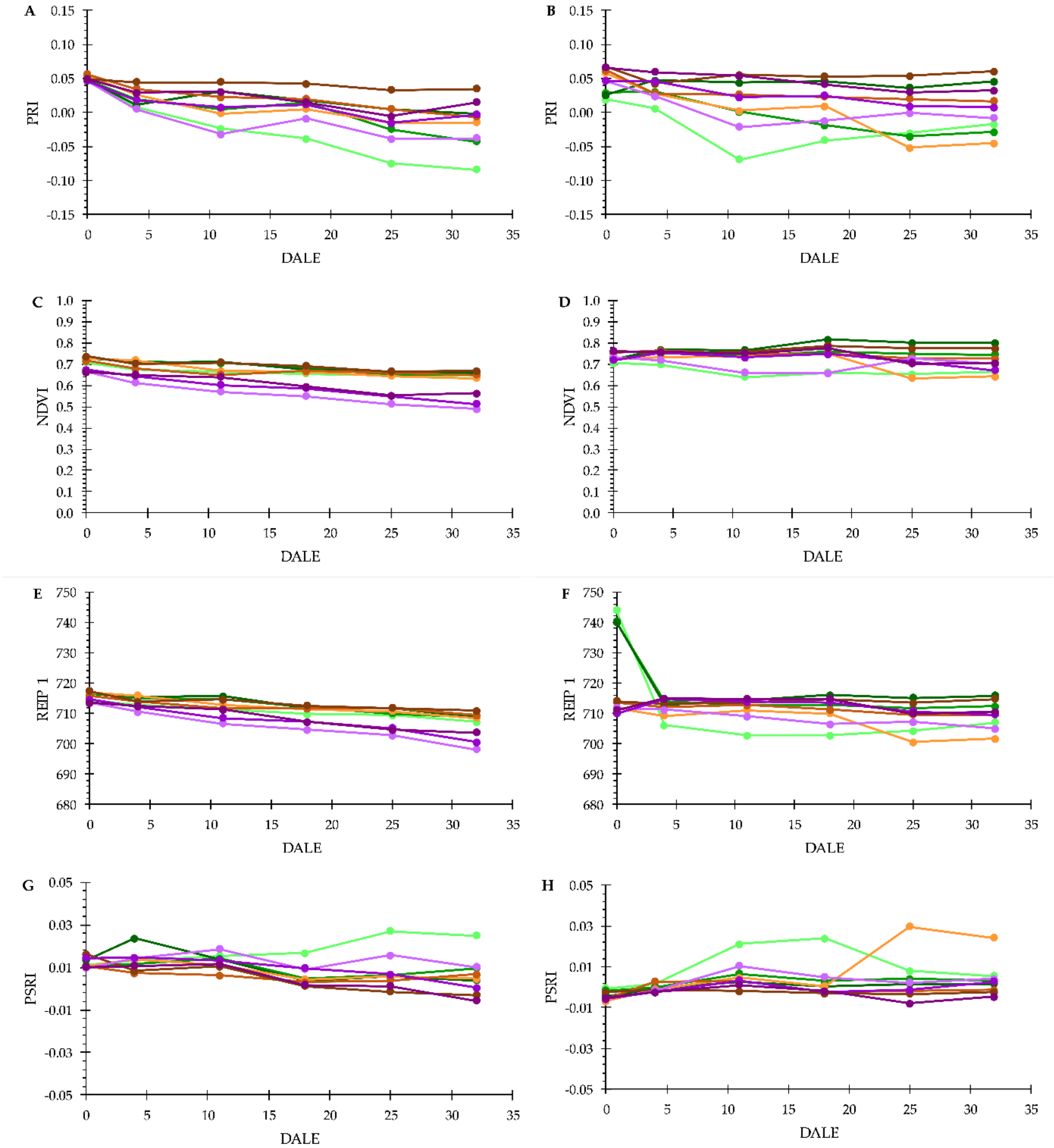


Figure 10. Performance-based vegetation indices for *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha', orange: 'Odoriko Amacha', purple: 'Amagi Amacha') under different shading conditions (dark coloring: full shade, medium coloring: partial shade, light coloring: no shade) in 2018 (A,C,E,G) 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 (Figure10 C,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 (Soheila, 2000). High amounts of UV-B radiation result in lower chlorophyll content (Kakani et al., 2004). 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 (Collins, 1978; Filella and Peñuelas, 1994). In addition, the red edge is affected by severe water deficiency (Filella and Peñuelas, 1994). 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 (Boochs et al., 1990). 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 (Kakani et al., 2004). Leaf senescence (as indicated by PSRI) defined by chlorophyll, leaf protein and nitrogen loss, as well as decreased photosynthesis (Noodén et al., 2004), 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 (Yeary and Fulcher, 2013) 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.

Chapter 3 – Proposing a chemometric Normalized Difference Phyllodulcin Index (cNDPI) for Phyllodulcin Synthesis Estimation

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Current state: Under Review

1. Introduction

Hydrangea macrophylla subsp. *serrata* has gained interest in the food industry and pharmacology due to the dihydroisocoumarin (DHC) content of hydrangenol (HG) and phyllodulcin (PD). PD is of interest for its uses in functional foods, pharmacological properties or its traditional use in Japan as a sweet seasoning (Kawamura et al., 2002). HG could be used in medicine (Kim et al., 2016a; Gho et al., 2019). *Hydrangea* L. belongs to the family of *Hydrangeaceae* within the dicotyledonous flowering plants (Takhtajan, 2009). The taxonomic classification of *H. macrophylla* subsp. s*errata* has been discussed controversly though. The name "*H. serrata*" might be justified on a morphological level (Reed and Rinehart, 2006) and is commonly used for simplicity (van Gelderen and van Gelderen, 2004). Some authors refer to the common name "Amacha" (Suzuki et al., 1977; Fujii and Yoshida, 2005), which could be misleading, because it is also describing the provenience of *Hydrangea* species, cultivars, as well as the product derived from the leaves. Tachibana et al. used the name "Sweet Hydrangea" based on its traditional use as sweet tea from fermented leaves (Tachibana et al., 1974). As shown in a previous study, cultivars of. *H. macrophylla* subsp. *serrata* differ in their DHC content (Moll et al., 2021b). For developing PD-rich cultivars, it is important to understand the effectiveness of PD production in plants and how this could be assessed, evaluated and described. For further exploitation of DHC it is crucial to analyze the content within hortensias in detail. To screen a large number of plants, destructive chemical analysis is often too time consuming to allow for fast decisions. Non-invasive phenotyping tools have been developed to quantify dynamic plant traits across scales based on sensor measurements like hyperspectral imaging (Cendrero-Mateo et al., 2017). In analogy to well established vegetation indices, we suggested and evaluated a **c**hemometric **N**ormalized **D**ifference **P**hyllodulcin **I**ndex (cNDPI). In the context of PD containing *Hydrangea* species we used the collective term "tea-hortensia" as previously introduced by Moll et al. (2021b).

1.1 Phyllodulcin and hydrangenol

In tea-hortensias, DHCs are synthesized via the shikimic acid pathway and coumaric acid. A specific polyketide synthase enables the mevalonate pathway with stilbenecarboxylates as intermediates (Kindl, 1971). PD is a product of the precursor molecule HG (Yagi et al., 1977). The DHC derivate PD is the main taste-influencing component in Amacha (in Japanese 甘い = amai = sweet, 茶 = cha = tea). This traditional tea is used during Hanamatsuri, the ceremonies surrounding the birthday of the Buddha (Yasuda et al., 2004). The characteristic taste of the tea arises from PD, that expresses a similar taste as dihydrochalcones (Shin et al., 1995). Besides the usage for tea, PD was investigated for being anti-fungal (Nakajima et al., 1979), anti-bacterial (Braca et al., 2012), anti-ulcer and anti-allergic (Yamahara et al., 1994), anti-inflammatory (Dilshara et al., 2013) and a treatment against malaria (Kamei et al., 2000). PD is a potent inhibitor for lipid peroxidation *in vitro* and on microsomal level (Yamahara et al., 1994). Additionally, PD might reduce obesity-related symptoms in mice (Kim et al., 2017; Kim et al., 2018). In bovine adrenocortical cells, PD was found to enhance cyclic AMP-induced steroidogenesis (Kawamura et al., 2002).

In tea-hortensias, HG and PD are present in glycosylated form. A targeted post-harvest management can increase the enzymatic activity for deglycosylation and therefore increase PD yield (Jung et al., 2016). Highest concentrations of HG were found in roots, which contained 2 to 3-fold more HG than flowers (Ibrahim and Towers, 1960). On the contrary, highest PD concentrations were found in sepals followed by buds and young leaves (Ujihara et al., 1995). A recent study found higher PD concentrations in young leaves compared to older leaves in different tea-hortensia cultivars grown under different cultivation conditions (Moll et al., 2021b). Furthermore, the PD content is affected by season. Highest concentrations were found in June, although plant’s characteristic PD contents were stable at different locations (Ujihara et al., 1995).

1.2 Evaluation by using indices

Description of different biological and biodiversity-related data can be performed by using indices (Davari et al., 2011), which can support decisions of scientists and political actors (Ribaudo et al., 2001). “Normalized Difference”-indices are characterized by the general formula

Index value = (a-b)/(a+b)

and are commonly used for a broad range of agricultural and environmental applications such as the widely used Normalized Difference Vegetation Index – NDVI (Rouse et al., 1974) or the

Normalized Difference Bareness Index – NDBaI (Zhao and Chen, 2005), which distinguishes areas with vegetation from areas with less or no vegetation, or the Normalized Difference Water Index – NDWI (Ji et al., 2009) for differentiation of land from water bodies. Also, different vegetation indices (VIs) used this formula structure to describe non-invasive qualitative assessment of plant traits, e.g. the Normalized Phaeophytinization Index – NPQI (Barnes et al., 1992), the Photochemical Reflectance Index – PRI (Gamon et al., 1992), the Normalized Pigment Chlorophyll Index – NPCI (Peñuelas et al., 1994), and the Structure Insensitive Pigment Index – SIPI (Peñuelas et al., 1995). Besides agricultural and environmental usage, indices are also found in chemometric analyses of plant-based (Riahi et al., 2009) and animal-based products (Cheng et al., 2016). As stated by Struber et al. (1999), the synergy of empirical breeding, marker-assisted selection, and genomics is a key for future breeding (Stuber et al., 1999). Nowadays, indices form a valid addition to improve plant breeding (Céron-Rojas and Crossa, 2018). They are widely used in non-invasive phenotyping methods on autonomous platforms like drones as a versatile tool to describe a large number of plant trait data under different conditions (Cendrero-Mateo et al., 2017), like growth or health (Thomas et al., 2018).

1.3 Aim of the study

We assumed that beside the total content of DHC in plants, the conversion efficiency of the precursor HG into PD can be used to distinguish cultivars and offspring plants. More efficient plants would be promising candidates for further breeding independent from the total content measured. We hypothesized that different tea-hortensia cultivars produce HG and PD at different ratios and that the ratio of HG:PD can be used as a chemometric marker for PD-synthesis as the PD content in plants seems to be more independent to external effects except seasonal changes. Based on former results obtained in studies presented in 1.1, PD synthesis seems to be mainly genetically determined. In this study we propose a new chemometric Normalized Difference Phyllodulcin Index (cNDPI) to classify PD-producing *Hydrangea* species based on HG-PD conversion efficiency which is a major driver of PD synthesis. Therefore, multiple sub-experiments (SEs) using the three tea-hortensia cultivars (‘Amagi Amacha’, ‘Oamacha’, and ‘Odoriko Amacha’) were re-evaluated to investigate the feasibility and reliability of such an index. In addition, we tested the robustness of this index using 50 plants of a F_1 population from a cross ‘Odoriko Amacha’ x ‘Yae Amacha’ with varying HG and PD contents.

2. Material and Methods

2.1 Plant material

The *H. macrophylla* subsp. *serrata* cultivars ‘Amagi Amacha’, ‘Oamacha’ and ‘Odoriko Amacha’ were used for all SEs. Plants were obtained as rooted cuttings from Kühne Jungpflanzen, Claus Kühne (Dresden, Germany) and Kötterheinrich Hortensienkulturen e.K. (Lengerich, Germany). After transplanting into new pots, plants were cultivated in a greenhouse until usage for the different SEs. Plant age, height, substrates and pot size varied depending on the specific SE. In addition, a F_1 population derived from a cross between the tea-hortensia cultivars ‘Odoriko Amacha’ and ‘Yae Amacha’ was investigated regarding cNDPI. F_1 plants were potted in 2018 in 3 L pots filled with Einheitserde ED73 (Einheitserdewerke Werkverband e.V., Sinntal-Altgronau, Germany) and were cultivated in a frost-free greenhouse as well as a Haygrove tunnel. All experiments were performed at Campus Klein-Altendorf in Rheinbach, Germany.

2.2 Data collection

The data used for index calculation was obtained in 12 sub-experiments (SE01–SE12) based on the cultivars ‘Amagi Amacha’, ‘Oamacha’ and ‘Odoriko Amacha’. The sub-experiments were performed from August 2018 until October 2020 to study the reaction of tea-hortensias to different abiotic stressors and the effect of harvest time, plant age and post-harvest treatments on HG and PD content. Each of these sub-experiments included these three cultivars. In total, 1,291 measurements based on leaf samples were obtained: 405 for ‘Oamacha’, 442 for ‘Odoriko Amacha’ and 444 for ‘Amagi Amacha’. Leaf samples of F_1 plants were harvested in June 2019 (E1), September 2019 (E2) and June 2020 (E3). In E1, plants were cultivated in a greenhouse, whereas the same plants were cultivated in a Haygrove tunnel in E2 and E3.

2.3 HG, PD and DHC quantification

The quantification of total HG and PD content (glycosides and their aglycones) was performed using a Waters Acquity UPLC® I-Class System. Sample preparation and HG, PD and DHC quantification were performed as described by Moll et al. (2021b).

2.4 Index calculation

For the calculation of the proposed chemometric Normalized Difference Phyllodulcin Index (cNDPI) the formula-structure “(a-b)/(a+b)” was used (see 1.2 evaluation by using indices). This operation leads to

cNDPI = (PD-HG)/(PD+HG)

with PD = Phyllodulcin [% DM] and HG = Hydrangenol [% DM]

resulting in index values between -1 and +1 where -1 indicates a plant only containing HG (no conversion into PD) and +1 a plant converting all HG into PD. For the calculations, all 1,291 measurements where included regardless of possible mistakes during data acquisition to prevent an overfitting of data by removing datapoints retrospectively. Additionally, the effect of treatment was not taken into consideration to ensure that the cNDPI would still classify cultivars correctly over a wide range of possible applications. For the breeding population, only F_1 plants were considered for the index calculation that produced PD in all three environments.

2.5 Statistical Analysis

Statistical analysis was performed using JMP Pro 16 (2021, SAS Institute Inc.). For the analysis of variance (ANOVA), the different genotypes were considered as fixed factors, while sub-experiments and the environments E1–E3 were considered as random factors. HG and PD contents were applied as metric values. Tukey-HSD was used as post-hoc test to determine homogenous subgroups at $\alpha = 0.05$. For the comparison of cNDPI values between cultivars, a linear discriminant analysis was performed for all SEs separately as well as the complete dataset over all SEs. To test for significant differences between environments in the F_1 population, a two-sided t-test with a Bonferroni correction ($p = 0.05/3$) was performed. A linear regression for the F_1 population was carried out to determine the predictability of HG, PD, DHC and cNDPI between different environments.

3. Results and Discussion

3.1 Hydrangenol and phyllodulcin content

The basis of the cNDPI calculation are the contents of HG and PD determined for each SE as well as the F_1 population for E1–E3. The cultivars investigated in this study showed significant differences in HG and PD content (Figure 1). Generally, the HG content was highest in ‘Odoriko Amacha’ and lowest in ‘Amagi Amacha’, whereas the PD content was highest in ‘Amagi Amacha’ and lowest in ‘Odoriko Amacha’. In contrast, ‘Oamacha’ showed medium values of both of them, HG and PD. Total DHC contents were significantly lower in ‘Oamacha’ compared to the other cultivars, whereas the total DHC contents of ‘Odoriko Amacha’ and ‘Amagi Amacha’ showed no significant differences. Significant differences between for HG, PD and DHC were observed between SEs in all three cultivars. These differences refer to different treatments and harvest dates as well as seasonal fluctuations during plant growth. These fluctuations were similar in all cultivars. Previous studies found seasonal differences in PD content as well as PD differences depending on the plant organ, the leaf age and shading regimes (Ujihara et al., 1995; Moll et al., 2021b). However, the effects of treatments were comparably low, supporting the hypothesis of genetics and harvest date being the main driver of PD synthesis.

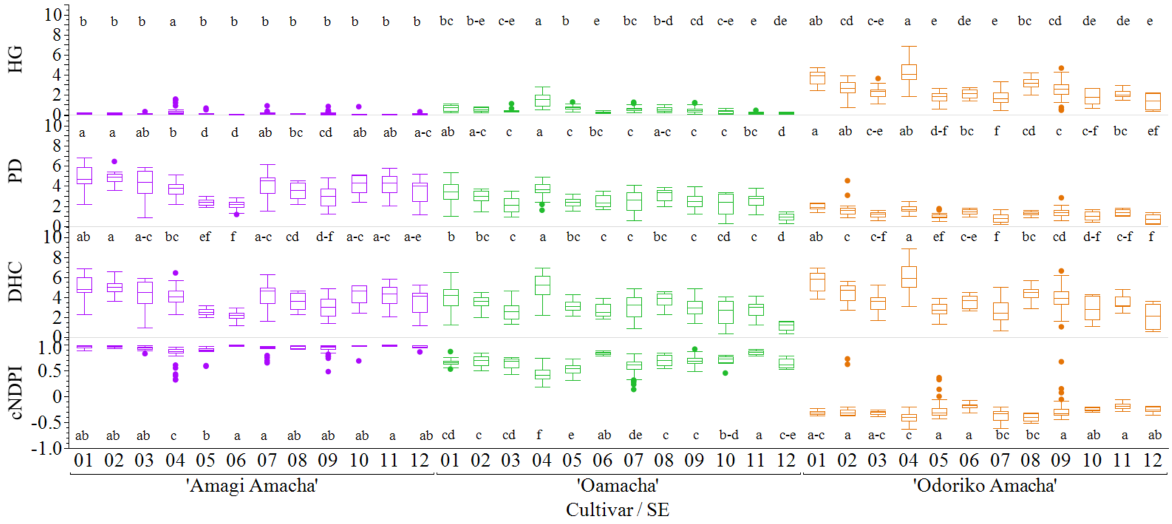

Figure 1. Hydrangenol (HG), phyllodulcin (PD), total dihydroisocoumarin (DHC) content and cNDPI in tea-hortensia cultivars for SE 01–SE12 assembled for cNDPI calculation. Significant differences between SEs for each constituent and cNDPI and cultivar were determined by ANOVA and Tukey-HSD ($p \leq 0.05$). Significant differences within a cultivar are indicated by letters a–f.

The analysis of the F_1 population showed that the PD contents of F_1 individuals ranged from 0.001% up to 6.916% with a mean PD content of 2.635% in E1, 1.809% in E2 and 2.223% in E3. Lower content of HG was found, ranging from 0.077% to 5.567%, with 1.738% in E1, 1.580% in E2 and 1.633% in E3. Over all environments, mean DHC content was 4.373% in E1, 3.390% in E2 and 3.861% in E3, ranging from 0.375% to 8.012%. The paired t-tests of the F_1 population revealed significant differences between environments in HG contents for all three environment-comparisons. Besides the comparison of E1 and E2, differences in PD and DHC content were significant as well. This differentiation of constituent contents fits the seasonal fluctuations stated earlier (1.1).

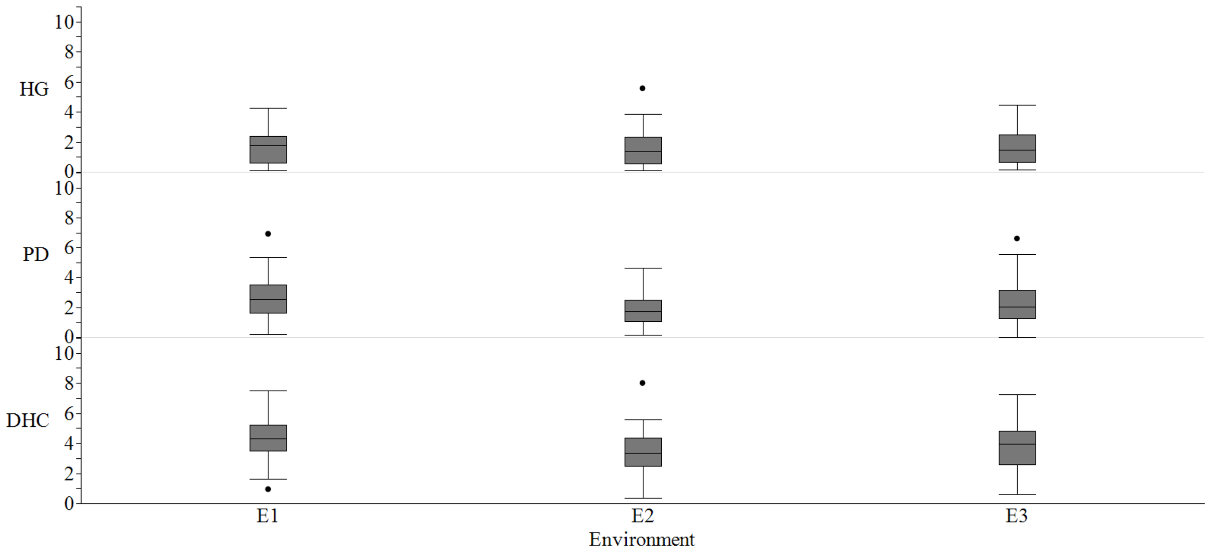

Figure 2. Hydrangenol (HG), phyllodulcin (PD) and total dihydroisocoumarin (DHC) content of 50 F_1 plants derived from a cross ‘Odoriko Amacha’ x ‘Yae Amacha’ determined in three different environments (E1–E3) assembled for cNDPI calculation.

3.2 Cultivar-specific cNDPI

The distribution of cNDPI values for each cultivar is shown in Figure 3. The cNDPI ranged from 0.318 to 0.999 in ‘Amagi Amacha’, from 0.133 to 0.915 in ‘Oamacha’, and -0.623 to 0.724 in ‘Odoriko Amacha’. ‘Amagi Amacha’ showed on average the highest cNDPI over all sub-experiments with a mean of 0.922 ± 0.083, followed by ‘Oamacha’ 0.618 ± 0.143 and ‘Odoriko Amacha’ -0.326 ± 0.147. cNDPI values do not overlap at 10% and 90% quantiles between the three cultivars, although outliers were included in the analysis. Some SEs forced extreme reactions of plants. Still, the cNDPI for each cultivar fits in a range that allows for a differentiation of cultivars. This shows the robustness of the index and its applicability.

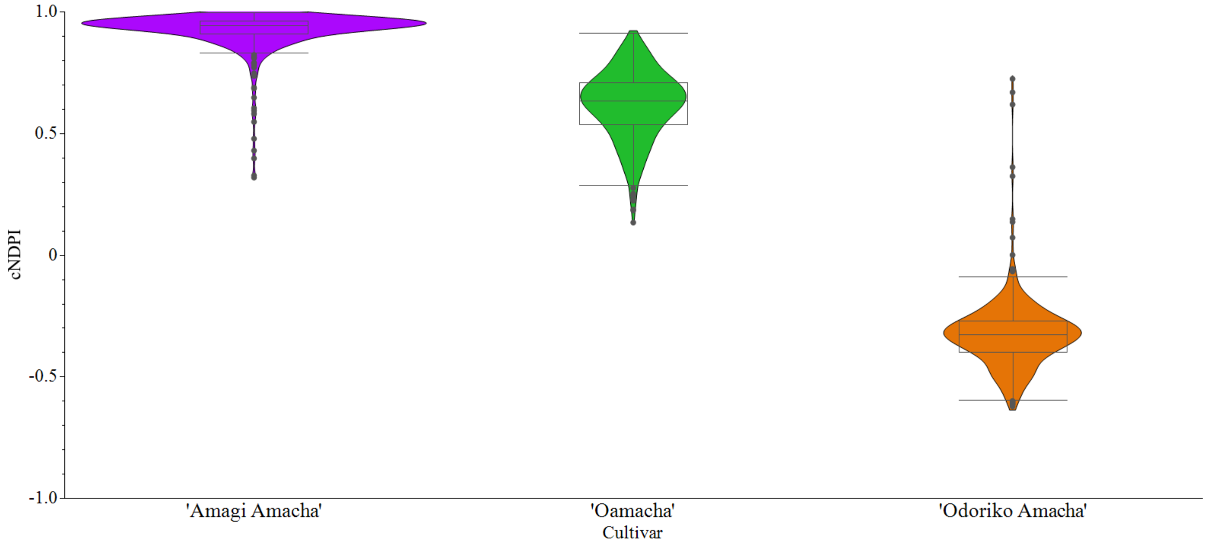

Figure 3. Violin plots of cNDPI for three *H. macrophylla* subsp. *serrata* cultivars 'Amagi Amacha', 'Oamacha', and 'Odoriko Amacha'.

The discriminant analysis showed that only 75 out of 1,291 (5.8%) cNDPI values assigned the cultivars incorrectly. This is mainly explained by overlapping values of 'Amagi Amacha' and 'Oamacha' and extreme values of 'Odoriko Amacha' as well as 'Amagi Amacha'. 'Oamacha' showed the most incorrect assignments with 53, followed by 'Amagi Amacha' with 16 and 'Odoriko Amacha' with 6. Nevertheless, 96% and 99% cNDPI values of 'Amagi Amacha' and 'Odoriko Amacha', respectively, were assigned correctly as well as 87% of 'Oamacha'. As multiple biotic and abiotic influences on plant performance were included via SEs, cNDPI showed to be a stable classification system for the three tea-hortensia cultivars as well as descriptor for HG–PD conversion.

Nevertheless, HG is a key factor for plant breeding, because it is the precursor of PD. In order to increase the PD content, plants with high cNDPI values might be crossed with HG rich plants to achieve PD rich offspring. Also, DHC rich plants might be used for breeding, because they contain the genetic resources to synthesize PD, besides high amounts of HG. Plants with a cNDPI value below 0 may not be suitable for plant breeding as they show a low conversion of HG to PD.

3.3 cNDPI in SEs

The range of cNDPI values differed partially between SEs most likely due to treatment-related or seasonal effects (e.g. SE06 and SE07). Nevertheless, the cNDPI allowed a resilient differentiation and identification of the three studied cultivars. The lowest and the highest 10% quantiles, respectively, were 0.792 (SE04) and 0.991 (SE06) for 'Amagi Amacha', 0.345 (SE04) and 0.907 (SE11) for ‘Oamacha’ and -0.566 (SE04) to 0.087 (SE06) for ‘Odoriko Amacha’ (Figure 4). The distribution of cNDPI values for every SE in relation to the corresponding contents of HG, PD and DHC is illustrated in Figure 1.

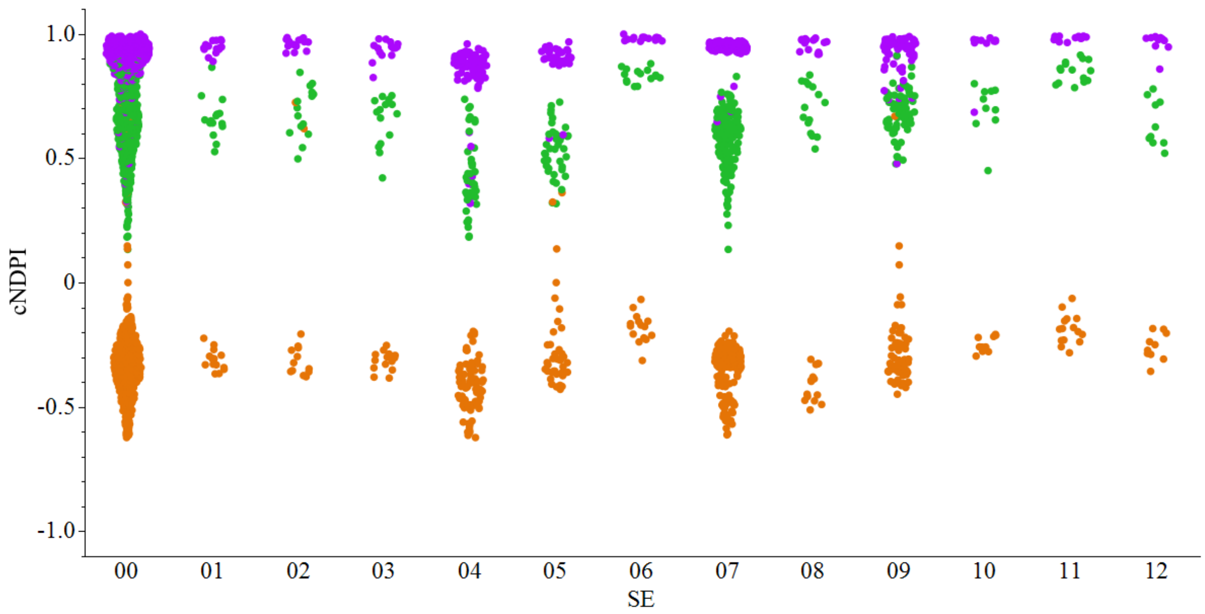

Figure 4. Distribution of cNDPI values for ‘Amagi Amacha’ (purple), ‘Odoriko Amacha’ (orange) and ‘Oamacha’ (green) based on 12 sub-experiments (SE01–SE12). SE00 marks the pooled data for all SEs.

The discriminant analysis showed that the percentage of correct assignments of cultivars by cNDPI was depending on the SE, with all SEs assigned at least 92.9% correctly. SE03, SE06, SE11 and SE12 showed 100% correct assignments. In SE01, SE05, SE07, SE08, SE10, 99.9–95% of the assignments were correct. In SE04 93.5%, SE02, 93.3% and SE09 92.9% of cultivars were grouped correctly. This data shows that cNDPI values of different cultivars were mostly stable over time and experiment. Therefore, this index is able to describe the genetically controlled synthesis of PD from HG unaffected by environmental fluctuations caused by e.g. season, management techniques or stress. Thus, the cNDPI provides a feasible and simple tool for the description of PD conversion efficiency from HG, and therefore, is a helpful selection criterion.

3.4 *cNDPI application in a segregating F_1 population*

As mentioned in 3.1 and shown in Figure 5, HG, PD and DHC contents of F_1 plants varied significantly between environments. Conversion of HG into PD, as described by cNDPI, is only significantly different between E2 and E3 (paired t-test: $p = 0.258$). Therefore, we assume that the HG, PD and DHC contents vary over time, whereas the conversion rate might be more stable. The correlation analysis confirms this assumption. Comparisons of HG, PD and DHC contents between environments resulted in R^2 values ranging from 0.3 to 0.7. Contrary, comparisons of cNDPI values between the environments resulted in $R^2 > 0.9$ in all three comparisons (Figure 5 J–L). These results strongly indicate that HG, PD and DHC contents vary, while the conversion rate is highly stable. Furthermore, cNDPI differences between environments were determined for each single F_1 plant. The cNDPI differences ranged between 0.008 and 0.552, on average 0.179 ± 0.111. 75% of plants showed cNDPI differences of maximum 0.227, emphasizing the stable, genotype-specific HG to PD conversion.

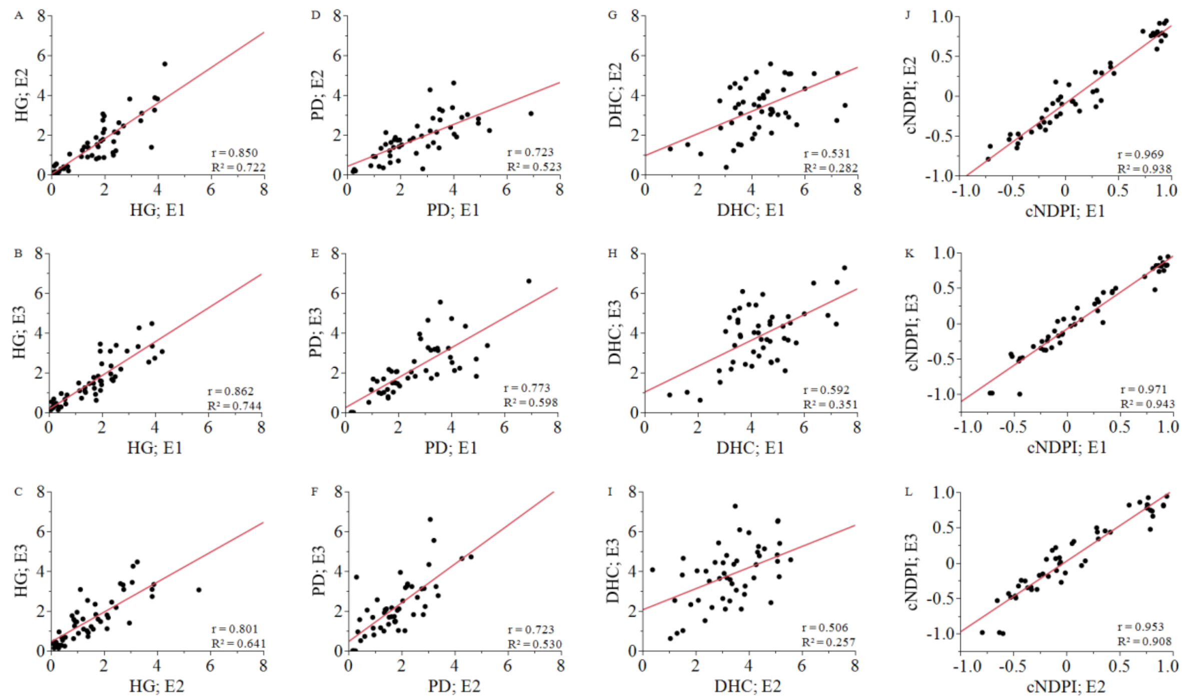

Figure 5. Correlation of HG, PD and DHC contents and cNDPI in three environments (E1–E3) based on 50 F_1 plants including correlation coefficients (r) and coefficients of determination (R^2). HG: hydrangenol (A–C); PD: phyllodulcin (D–F); DHC: dihydroisocoumarin (G–I); cNDPI: chemometric Normalized Difference Phyllodulcin Index (J–L). E1 ~ E2: A, D, G, J; E1 ~ E3: B, E, H, K; E2 ~ E3: C, F, I, L.

4. Conclusions

The proposed **c**hemometric **N**ormalized **D**ifference **P**hyllodulcin **I**ndex (cNDPI) provides a feasible and simple tool for the description of PD conversion efficiency from HG. In targeted plant breeding, it will support the selection of plants with a high PD conversion efficiency. The results of this study demonstrate that the cNDPI allows a significant and reliable differentiation of the tea-hortensia cultivars ‘Amagi Amacha’, ‘Odoriko Amacha’ and ‘Oamacha’ under different experimental settings. The results indicate, that cNDPI values >0.9 seem reasonable as a target value while cNDPI values up to 0.999 were achieved by ‘Amagi Amacha’. Based on the results, the index can be used in plant breeding as a chemometric marker to select plants with an efficient PD synthesis. Besides PD synthesis efficiency, HG seems to be a limiting factor in PD synthesis as well. As long as the genetics of PD synthesis as well as precise synthesis pathway are unclear, the cNDPI provides a promising tool for description of HG-to-PD conversion. In future studies, it is intended to combine the cNDPI with hyperspectral high-throughput phenotyping of tea-hortensias to predict DHC contents in plants by non-invasive methods.

Chapter 4 – VIS-NIR Modeling of Hydrangenol and Phyllodulcin Contents in Tea-Hortensia (*Hydrangea macrophylla* subsp. *serrata*)

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1. Introduction

Hydrangea macrophylla subsp. *serrata* has shown to be of interest for containing dihydroisocoumarin (DHC) glycosides hydrangenol (HG) and phyllodulcin (PD). Synthesis of DHCs in *H. macrophylla* subsp. *serrata* are driven by the shikimic pathway and coumaric acid. Intermediates during synthesis are stilbenecarboxylates in the mevalonate pathway that is enabled by a specific polyketide synthase (Kindl, 1971). During synthesis of PD, HG functions as a precursor (Yagi et al., 1977). HG and PD contents are influenced by seasonal changes and leaf age and seem to be predetermined by genetics than environment (Ujihara et al., 1995; Moll et al., 2021b). The popular name for *H. macrophylla* subsp. *serrata* "tea-hortensia", as proposed by Moll et al. (Moll et al., 2021b), is based on the plants original use for ceremonial tea. The tradition of the Hanamatsuri, the ceremonies surrounding the birthday of the Buddha, use the tea from fermented and dried leaves of *H. macrophylla* subsp. *serrata* (Suzuki et al., 1978). The characteristic taste of Amacha (Japanese for 甘い = amai = sweet, 茶 = cha = tea) is caused by PD (Shin et al., 1995). In addition to the characteristic taste, HG and PD provide multiple positive benefits. Besides others, HG enhances the growth-promoting activity from gibberellin (Asen et al., 1960). Analogue to PD, HG shows anti-fungal effects (Nozawa et al., 1981), but also affects the activation of Hyaluronidase and possesses anti-allergic activities (Kakegawa et al., 1988). In BV-2 microglial cells, HG inhibits the lipopolysaccharide-induced nitric oxide production (Kim et al., 2016a). Inhibition of proliferation, migration and invasion of EJ bladder cancer was investigated for HG (Shin et al., 2018) as well as UV-induced skin damage reduction in mice (Myung et al., 2019). Leaf extracts from *Hydrangea macrophylla* L. showed to be a potent fertilizer for mungbean – *Vigna radiata* L. (Sanjeewani et al., 2019). Beneficial activities of PD include being anti-fungal (Nakajima et al., 1979), anti-ulcer and anti-allergic (Yamahara et al., 1994), anti-bacterial (Braca et al., 2012) and anti-inflammatory (Dilshara et al., 2013). In vitro and on a microsomal level, PD is a potent inhibitor for lipid peroxidation (Yamahara et al., 1994). Additionally, further animal experiments investigated enhancement of cyclic AMP-induced steroidogenesis in bovine adrenocortical cells (Kawamura et al., 2002) while obesity-related symptoms in mice could be reduced (Kim et al., 2017; Kim et al., 2018). Positive effects of PD as a malaria treatment were investigated as well (Kamei et al., 2000).

Until now, quantification of HG and PD is performed using Ultra Performance Liquid Chromatography (UPLC), with one possible protocol introduced by Moll et al. (2021). While providing advantages in comparison to conventional HPLC, the analysis can be time consuming, depending on the specific compounds (Swartz, 2005), even if UPLC showed to be up to nine times faster than HPLC (Nováková et al., 2006). Besides being advantageous in comparison to other techniques (Kumar et al., 2012), UPLC is not suitable to be performed by farmers for real-time estimations in field for PD content quantifications or prediction.

Hyperspectral modeling of the VIS-NIR and NIR regions showed to be able to model leaf constituents in natural products (Cozzolino, 2009) over a wide range of plants (coffee, tea, cocoa, tobacco) and spices, medicinal and aromatic plants in general (Schulz, 2004) or the shelf life of products (Giovenzana et al., 2014). Additionally, the discrimination of plants using a NIRS-based chemometric evaluation presents a promising field of research (Ercioglu et al., 2018a). On a leaf-based level, contents of flavonoids (Wulandari et al., 2020) or terpenoids (Ercioglu et al., 2018b) were investigated using infrared spectroscopy and chemometrics. NIR modeling is used for modeling volatile organic compounds (VOC) in *Mentha* (Schulz et al., 1999). Besides constituent analysis and estimation, yield presents a field of research as well, for instance in alfalfa - *Medicago sativa* L. (Noland et al., 2018) or wheat - *Triticum aestivum* (Pantazi et al., 2016). For rice yield, the possibilities of multispectral data from drones was investigated as well (Stroppiana et al., 2015).

In the system of hyperspectral measurements, three factors influence the data acquisition: the scene (the observable space in front of the spectrometer), the sensor system, and the processing system (Landgrebe, 1997). Portable devices allow for satisfying results from VIS-NIR spectra as well (Giovenzana et al., 2014).

We hypothesized that measurement conditions do not influence model accuracy, and that it is possible to use VIS-NIR spectrometers to model for HG and PD contents. Based on these results the suitability of models based on the handheld PolyPen RP400 is to be investigated as a simple and fast proxy for HG and PD contents on a field scale. This would enable farmers to find optimized harvest dates during the season and acts as a decision support in tea-hortensia cultivation.

This article provides the first assessment of hyperspectral modeling as a decision support for farmers as well as for scientific purposes in the field of tea-hortensias and the dihydroisocoumarins hydrangenol and phyllodulcin. Seasonal changes (Ujihara et al., 1995) lead to uncertainties of ideal harvest time, as peaks in PD accumulation might differ between cultivars. Therefore, a fast quantification of HG and PD is needed on a field-scale. Additionally, the study further investigated the possibilities of cultivar differentiation as well as the impact of measurement condition on model quality.

2. Materials and methods

2.2 Spectrometer set-up

Multiple set-ups of spectrometers were used for the experiments presented in this study. First (in 2019), two spectrometers from Ocean Insight (Orlando, FL, USA) were used, namely the "Red Tide USB650 Fiber Optic Spectrometer" (350-1,000 nm), from now on shortened to "Red Tide", and the "Flame-NIR Miniature Spectrometer" (950-1,650 nm), in this study shortened to "Flame-NIR". Red Tide provides a resolution of 651 pixels per spectrum (2 nm Full-Width at Half-Maximum (FWHM)), equal to one datapoint per nm with a signal-to-noise ratio of 250:1. The Flame-NIR signal-to-noise ratio is 6,000:1 providing 128 datapoints for the spectrum with a resolution of 10 nm FWHM. Both spectrometers were connected to an external Ocean Insight Tungsten Halogen Light Source HL-2000-HP using a QR200-12-MIXED fiber optic. "SMA connector" was inserted at maximum and the "Attenuator" was pulled out at maximum. The probe was fixated 1 mm above the sample at an angle of 90° to leaf surfaces (specular reflectance) using an RPH-1 reflection probe holder. Spectra were collected in reflectance mode and calibration was performed using the Spectroscopy Application Wizard build into the OceanView 1.6.7 software and using a WS1-Diffuse Reflectance Standard. In the first experiment, the spectrometers were used separately measuring four spots per leaf. Integration time was set to 50 ms and 733 ms for Red Tide and Flame-NIR respectively. Additionally, both spectrometers were used with 2 scans to average and a boxcar width of 5. Spectra of the four scanning spots were then averaged for further calculations.

In the second experiment (in 2021) the spectrometers were used simultaneously to measure the same spot on a sample. Integration time was set to 6 ms for Red Tide with 25 scans to average and 320 ms for Flame-NIR with 5 scans to average. Again, a boxcar width of 5 was adjusted. Re-calibration was done after about 20 to 30 measurements storing a white reference and a dark spectrum. Data collection and processing was performed using OceanView 1.6.7 and Microsoft Excel 2019.

Besides stationary spectrometers, the handheld PolyPen RP400 (UV-VIS), from now on "PolyPen", by Photon Systems Instruments (Brno, Czech Republic) covering a detection range from 380-780 nm with 256 datapoints and a resolution of 8 nm Half-Width at Half-Maximum was used to investigate reflectance spectra. Data collection and processing for the PolyPen was performed via SpectraPen 1.0.0.5 and Microsoft Excel 2019.

2.3 Plant material and cultivation

Plant material was obtained from commercial breeders for both experiments. For the first experiment Kühne Jungpflanzen, Claus Kühne (Dresden, Germany) delivered the plants, and for the second experiment Kötterheinrich Hortensienkulturen e.K. (Lengerich, Germany). Plant material for the first measurements was delivered to the Institute of Crop Science and Resource Conservation – renewable resources in May 2019 as rooted cuttings in 6 x 8 multipot trays and potted in Jiffy-Pots #130 (Jiffy Products International BV, Zwijndrecht, Netherlands) in June 2019. Until measurement, all plants were cultivated under the same conditions in a greenhouse and irrigated daily. A total of 5,000 plants per tea-hortensia cultivar ‘Amagi Amacha’, ‘Oamacha’ and ‘Odoriko Amacha’ were cultivated in this regime. In May 2019 plants from the same production cycle were delivered to Symrise AG (Holzminden, Germany) as well. Those plants from tea-hortensia cultivar ‘Oamacha’ were planted as part of a small exhibition garden.

2.4 Experimental design

The study comprised two separate measurement dates. The first measurement was part of an experiment carried out on July 9th 2019 to gain understanding of measurement specifics. Therefore, three tea-hortensia cultivars (‘Amagi Amacha’, ’Oamacha’, ‘Odoriko Amacha’) were analyzed using a black and a white background plate separately. Additionally, fresh leaves were measured after harvest and after drying at 40°C for 72 hours. Sample selection was performed by randomly taking 20 plants from a plant stock consisting of 5,000 plants per cultivar and measuring both upper fully developed leaves for each background and leaf water status.

Based on the first results a second trial was performed. The second block of measurements was part of an experiment carried out on August 20th in 2021 using in-field cultivated *H. macrophylla* subsp. *serrata* ‘Oamacha’, again using Red Tide and Flame-NIR. In this trial a total of 304 single fully developed upper leaves were taken to be measured as fresh leaves on a black background. To avoid wilting, 30 to 40 samples were collected immediately before data acquisition, so that the time slot between harvest and measurement was no longer than 30 minutes. Sample selection again was carried out by randomly taking leaves from in-plants. Reflectance spectra from 61 of these leaves were additionally taken using the PolyPen whereby the mean value of four measurements was calculated.

2.5 *Analysis of hydrangenol and phyllodulcin*

The analysis of hydrangenol and phyllodulcin was performed at Symrise AG (Holzminden, Germany) on a Waters Acquity UPLC® I-Class. An Acquity UPLC ελ PDA detector combined with a commercially available reversed phase C18 column (Luna Omega 1.6 µm Polar C18 50 x 2.1 mm) was used. The procedure was performed according to the protocol for hydrangenol and phyllodulcin quantification for tea-hortensias (Moll et al., 2021b).

2.6 *Outlier detection and spectra pre-processing*

Errors in a dataset, disturbing effects like spectral noise and many other factors influence data analysis and can affect model quality, thus data pre-processing has proven to be advisable (Famili et al., 1997). First, DHC contents (HG and PD) were checked for outliers. Samples with values exceeding three times the interquartile range of the 0.25 tail quantile were excluded from further calculations. Furthermore, the raw spectra were graphically examined and samples with curves strongly shifted on the x-axis were removed manually. In addition, a penalized basis spline (P-Spline) model, which minimizes residuals and avoids overfitting at the same time (Aguilera and Aguilera-Morillo, 2013; Aguilera-Morillo and Aguilera, 2015), was fitted to the reflectance measurements to detect multivariate outliers within the score plot of the first and second principal components. To get a first overview, models were developed using leave-one-out cross validation. Hereby the model is developed with n-1 training data (n corresponds to the number of samples) and the holdout sample is used for calibration. This process is repeated n times (Haaland and Thomas, 1988). After cross validation, another sample was discarded due to a high Hotellings T^2-statistic ($\alpha = 0.05$). This method is suitable for correlated variables and is considered as enhancement of Student's t-distribution for multivariate data sets (Hotelling, 1931). The procedures resulted in 40 spectra for 'Oamacha' and 'Odoriko Amacha' and 35 for 'Amagi Amacha' in 2019, as well as 296 spectra for the Ocean Insight spectrometers and 60 spectra for modeling with the PolyPen data in 2021.

Standard Normal Variate (SNV) transformation and Savitzky-Golay (SG) filters including smoothing, or first- and second order derivatives were the pre-processing methods in this study. SNV transformation corrects slope variations and scatter effects by first centering the individual spectra and then dividing them by their standard deviation (Barnes et al., 1989). Smoothing methods are suitable for reducing spectral noise (Barak, 1995). Based on the moving average method, polynomial smoothing, also known as Savitzky-Golay (SG) smoothing, fits a polynomial function to the data of a chosen smoothing interval so that these are entered with different weighting in the calculation of the value to be smoothed. This preserves the structure and spectral information even with larger smoothing intervals (Savitzky and Golay, 1964). In addition, the aligned SG polynomial functions fitted to the smoothing intervals can be derived (Luo et al., 2005). The obtained derivatives of the spectra remove baseline shifts (O'Haver, 1979). The optimal preprocessing methods have to be adapted individually for each model (Barbin et al., 2012). Here, it was found that SNV transformation before SG processing showed the best results. Due to strong noise (graphical inspection) of the SNV transformed spectra of the Red Tide spectrometer in 2021, the wavelength range below 400 nm was excluded from calculations. Afterwards, SG algorithms with 2nd to 8th degree polynomials with window points ranging from 11 up to 61 were tested. A summary of the methods and wavelength ranges used for modeling can be found in Table 1. Pretreatments were conducted in JMP Pro 16.

Table 1. Wavelength ranges and preprocessing methods (Standard Normal Variate (SNV) transformation and Savitzky-Golay (SG) filters) used for modeling of hydrangenol (HG) and phyllodulcin (PD).

Spectrometer (Year of experiment)	Wavelength range [nm] for SNV transformation	Wavelength range [nm] for SG filter	Preprocessing method (DHC compound)
Red Tide (2019)	350–938	360–928	SNV + SG smoothing: 5th polynomial order; distance to right/left filter edge = 10 (HG, PD)
Red Tide (2021)	350–1,000	420–918	SNV + SG smoothing: 5th polynomial order, distance to right/left filter edge = 20 (HG, PD)
Flame-NIR (2021)	940–1,664	-	SNV
Red Tide + Flame-NIR (2021)	400–1,664	-	SNV
PolyPen (2021)	325–792	353–765	SNV + SG 2nd derivative: 7th polynomial order; distance to right/left filter edge = 15 (HG)
		382–740	SNV + SG 1st derivative: 7th polynomial order; distance to right/left filter edge = 30 (PD)

2.7 Model development

Models were implemented in JMP Pro 16 (SAS) by Partial Least Squares Regression (PLSR). PLSR has proven to be a practical and widely used tool for investigating spectroscopic data

(Aleixandre-Tudo et al., 2015; Huang et al., 2020; Sanaeifar et al., 2020). In contrast to Multiple Linear Regression (MLR), this method enables processing of a large number of X-variables (here the wavelengths, also "predictors" or **X**), which can be intercorrelated and sometimes show strong noise (Wold et al., 2001). The aim is to establish a relationship between the predictors and at least one Y-variable (here the HG or PD content, also "response" or **y**), which allows predictions of the latter (Dunn et al., 1984). Performing a principal component analysis of both the predictors and responses and intercorrelating them reduces the number of variables to a few PLS principal components (also "factors" or "latent variables"), which simplifies modeling and separates relevant information (Geladi and Kowalski, 1986). In order to determine the appropriate number of factors and prevent overfitting or underfitting, validation of the model is required (Haaland and Thomas, 1988). Therefore, the samples were randomly split into three subsets: training, validation and test that will later result in a calibration, validation, and prediction model. The training set (composed of 70% of the samples) was used to estimate calibration model parameters, the validation set (20%) was used within the algorithm to optimize the calibration model, whose ability to predict was assessed with the separate test set (10%) not involved in the model building process. For calibration model development, data were scaled to unit variances (standard deviation of 1 by dividing the data by their variables standard deviation) and centered to mean value zero (by subtracting variables average values from the data) (Wold et al., 2001). Subsequently, all variables had the same impact on modeling in terms of variation and interpretation was simplified. NIPALS (Non-linear Iterative Partial Least Squares) was chosen as the algorithm. As Hu et al. (2020) reported, model performance can be affected by variable selection. So-called Variable Influence on Projection (VIP) (Wold et al., 1993) values describe the importance of a variable (x) in explaining both **X** and **y** (Wold et al., 2001). According to Wold et al. variables below a threshold of 1.0 can be removed. Models in this study were pruned until all variables were weighted with a factor greater than 0.8 (Wold et al., 1993).

2.8 Statistical Analysis

Parameters based on the determination of so-called residuals (e_i) can be used to assess the goodness of fit. Residuals are defined as the difference between the actually measured (y_i) and predicted analyte concentrations ($\hat{y}_i$) and represent the remaining variance in the data that cannot be explained by the principal components (Wold et al., 2001). In this study, both Root Mean Square Error (RMSE) as well as the coefficient of determination (R^2) were specified for the different subsets: training set, which equals to the calibration model (R_c^2 and RMSEC); validation set (R_v^2 and RMSEV); test set, which equal to the prediction set in common terminology

(R_p^2 and RMSEP) and the overall model (R_{total}^2 and $RMSE_{total}$). Equations are given in (1) and (2). Models with a low RMSE/root mean PRESS and a high R^2 score were considered to be good.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \quad (1)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (2)$$

n = number of samples (spectra), y_i = measured reference value of the sample i, $\hat{y}$ = predicted value of the sample i, $\bar{y}$ = mean value of all samples

Statistical analysis of HG and PD contents in the three cultivars and comparison of models R^2 regarding the spectrometers used and the partition of the sample sets was performed via Analysis of Variance (ANOVA) with Tukey-HSD as post hoc procedure to determine homogenous subgroups at a p-value of $p \leq 0.05$. Differences between 'Oamacha' datasets for Red Tide + Flame-NIR and PolyPen were investigated via t-test ($p \leq 0.05$) with significant differences marked with asterisk (*).

For the detection of cultivars via hyperspectral models and the impact of measurement background, a discriminant analysis was performed using the cultivars, leaf water status or backgrounds as category and HG, PD and both simultaneously from UPLC analysis as well as prediction models as covariables.

3. Results

3.1. Hydrangenol and phyllodulcin contents

The analysis of hydrangenol and phyllodulcin revealed significant differences between cultivars in 2019. HG contents were highest in 'Odoriko Amacha' (4.787% ± 1.066%), followed by 'Oamacha' (1.514% ± 0.649%) and 'Amagi Amacha' (0.293% ± 0.142%). Phyllodulcin did not show significant differences between 'Oamacha' (3.642% ± 0.692%) and 'Amagi Amacha' (3.906% ± 0.480%). Both cultivars yielded higher contents of PD than cultivar 'Odoriko Amacha' (1.794% ± 0.323%). In 2021 only 'Oamacha' was investigated. For both spectrometer setups used (Red Tide + Flame-NIR and PolyPen), different samples were taken out of the modeling process as described in the Material and Methods part regarding data pre-treatment and data elimination. Therefore, different means for HG and PD contents were calculated. For trial I (Red Tide + Flame-NIR) a mean HG content of 0.515% ± 0.133% and a mean PD content of 4.409% ± 0.264% were recorded. The analysis for Trial II (PolyPen) revealed 0.545% ± 0.145%

HG and 4.448% ± 0.270% PD. The statistical analysis determined no significant differences in mean constituent contents for HG and PD in 'Oamacha' that could be a result of different samples taken out of the calculations. The distribution of HG and PD contents by harvest year, trial and cultivar is illustrated in Figure 1.

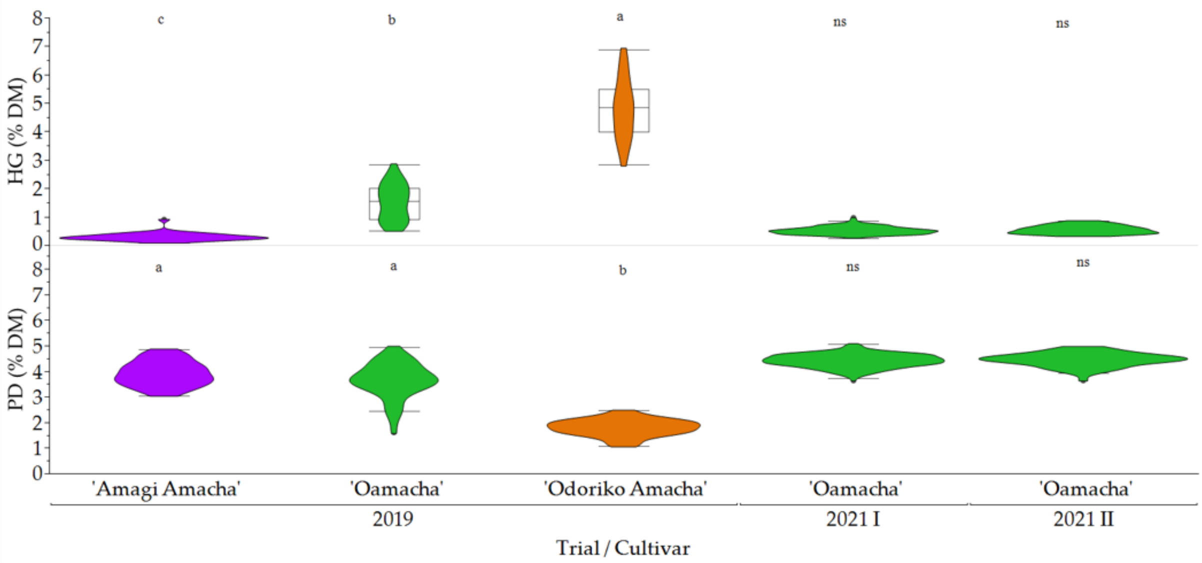

Figure 1. Hydrangenol (HG) and phyllodulcin (PD) contents of three tea-hortensia cultivars harvested on 9th July 2019 (Trial 2019) and 20th August 2021 (Trial 2021 I and 2021 II). Trial 2021 I represents data used for modeling based on the Ocean Insight spectrometers, while trial 2021 II indicates HG and PD contents for modeling PolyPen data. Differences in data used for models in 2021 occur according to data preprocessing described in 2. Material and Methods. Significant differences were determined by ANOVA and Tukey-HSD as post-hoc procedure ($p = 0.05$) and are indicated by letters (a–c) for 2019 while trials in 2021 were investigated by t-test ($p = 0.05$) with significant differences indicated by asterisks (*).

3.2. Differentiation of Cultivars

The first objective in modeling HG and PD from tea-hortensia cultivars was the differentiation of 'Amagi Amacha', 'Oamacha' and 'Odoriko Amacha'. Basic observation of the reflectance spectra (Figure 2) in combination with knowledge of HG and PD contents did not allow for a simple solution. Therefore, a linear regression of predicted content values in comparison to measured values was performed showing a strong relationship (Figure 3). Prediction of HG and PD was performed according to Table 1, using the Red Tide (2019) dataset. Statistical indicators for regression quality are shown in Table 2 in comparison to the other model set-ups in this study. For all three R^2 calculations (training, validation and test set) $R_{total}^2 > 0.9$ was calculated for HG, resulting in $R_p^2 = 0.998$ for the test set. Similarly, $R_p^2 = 0.910$ for PD was calculated for the test set. RSMEP for HG resulted in 0.116 while higher values for PD were calculated with RMSEP = 0.340.

Hydrangenol seems to be a reasonable component for classification of cultivars. Based on the UPLC analytics, 90.4% of samples are classified correctly for all samples taken into the experiments. In this, ‘Amagi Amacha’ showed 100%, ‘Odoriko Amacha’ 95%, and ‘Oamacha’ 77.5% accuracy. Usage of modeled HG contents seems to be reasonable as well, as 91.6% of samples were classified correctly. ‘Oamacha’ and ‘Odoriko Amacha’ were classified correctly in 100% of samples. ‘Amagi Amacha’ showed 75% accuracy.

The discriminant analysis revealed that predicted PD seems to be prone to errors when it comes to accessing cultivars correctly. Still, 29.6% of false classifications fall into the same range of wrong classifications based on predicted values in comparison to 32.2% when grouped according to actually measured PD contents in the lab. The overlap of ‘Amagi Amacha’ and ‘Oamacha’ in PD contents resulted in a high overlap in cultivar classification as well. ‘Odoriko Amacha’ is classified correctly for 100% of inputs based on lab analysis as well as modeled PD. 80% of PD is classified correctly when only the test set is observed, again being explained by the overlap of actual PD contents measured via UPLC.

Based on lab analytics, the combination of HG and PD as covariables for classification of cultivars showed the highest accuracy, with 93% of correct classifications. No false classification was observed in ‘Odoriko Amacha’, one wrong grouping in ‘Amagi Amacha’ (97% accuracy) and 82.5% accuracy for ‘Oamacha’.

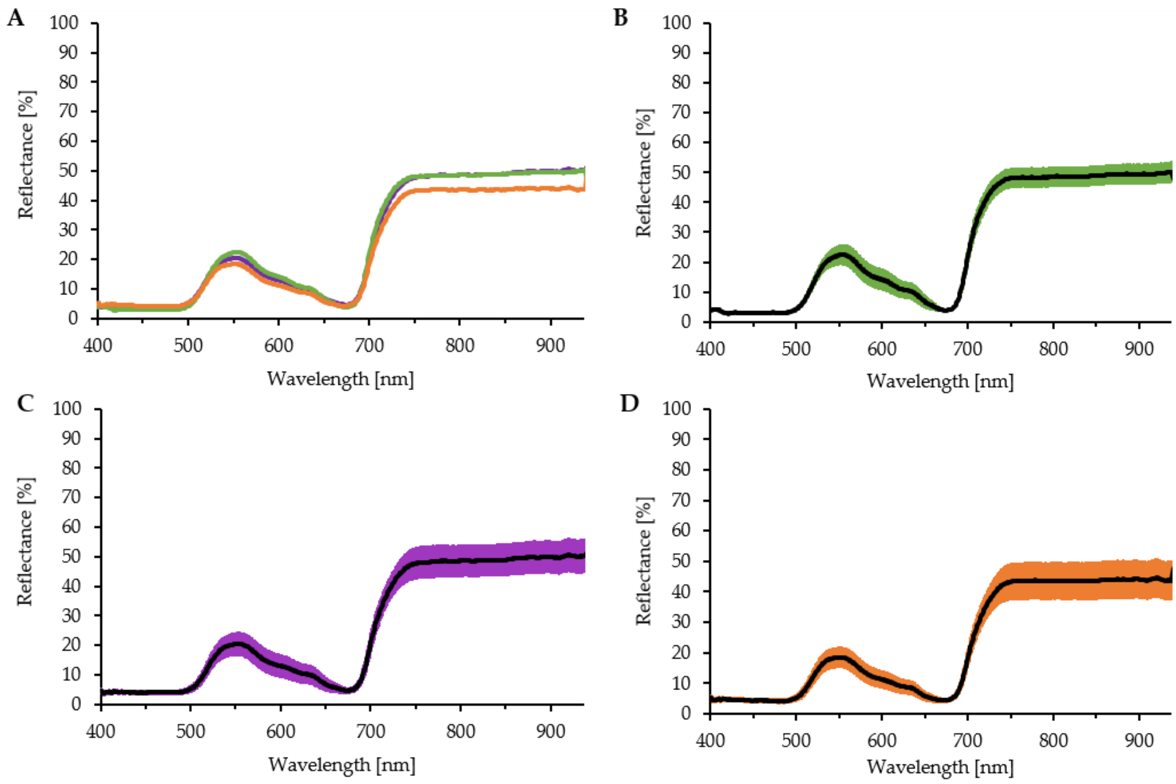

Figure 2. Averaged raw spectra of *H. macrophylla* subsp. *serrata* cultivars (green: 'Oamacha' (n = 40), orange: 'Odoriko Amacha' (n = 40), purple: 'Amagi Amacha' (n = 35)) measured with Red Tide spectrometer in 2019 using fresh leaf samples and a black measuring background. **A** shows the averaged spectra while **B–D** show the average value ± SD of the respective cultivar.

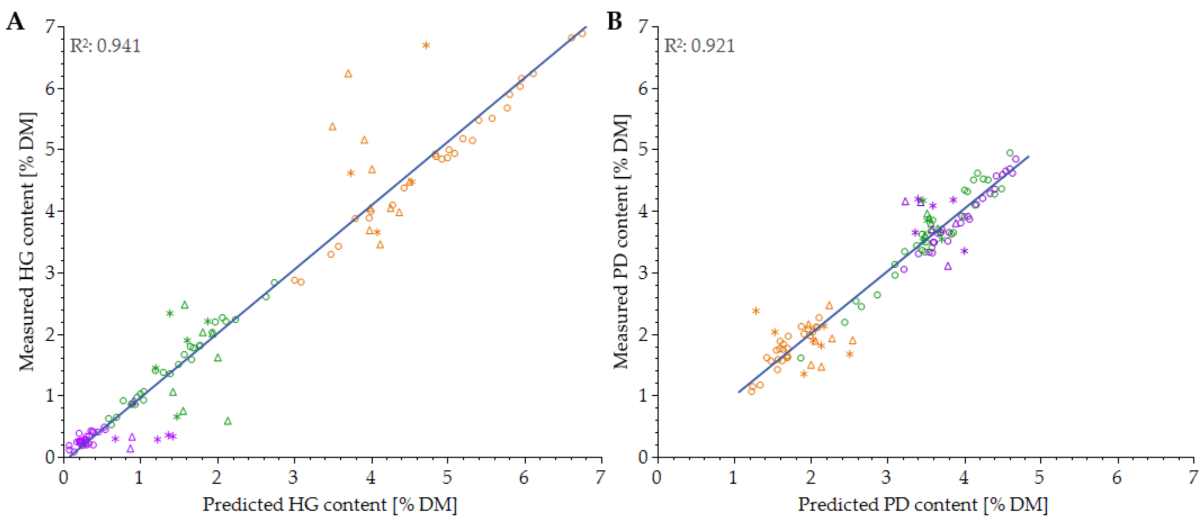

Figure 3. Regression of predicted and measured (Red Tide, 2019) contents of HG (**A**) and PD (**B**) for three tea-hortensia cultivars (purple: 'Amagi Amacha'; green: 'Oamacha'; orange: 'Odoriko Amacha') including coefficient of determination. Different symbols indicate the training (o), validation (Δ) and test (*) set (HG: training (n = 86), validation (n = 18), test (n = 11); PD: training (n = 80), validation (n = 26), test (n = 9)).

3.3 *Impact of measurement conditions*

For the transferability and replicability of the models for tea-hortensia cultivar differentiation as well as HG or PD assessment, the influence of measurement background and leaf water status was investigated. While reflectance spectra of ‘Oamacha’ (Figure 4) showed obvious differences in curve structure, the influence of leaf water status and background color on calculated regressions was neglectable. Background color influenced the shape of reflectance spectra in tea-hortensia cultivar ‘Oamacha’, with higher reflectance on white backgrounds in comparison to black ones for fresh and dried leaves. In the range from 400-700 nm effects of measurement background were more pronounced for fresh leaves than for dried leaves. Contrary, in the region of red-edge until 1,000 nm, differences were larger for dried leaves.

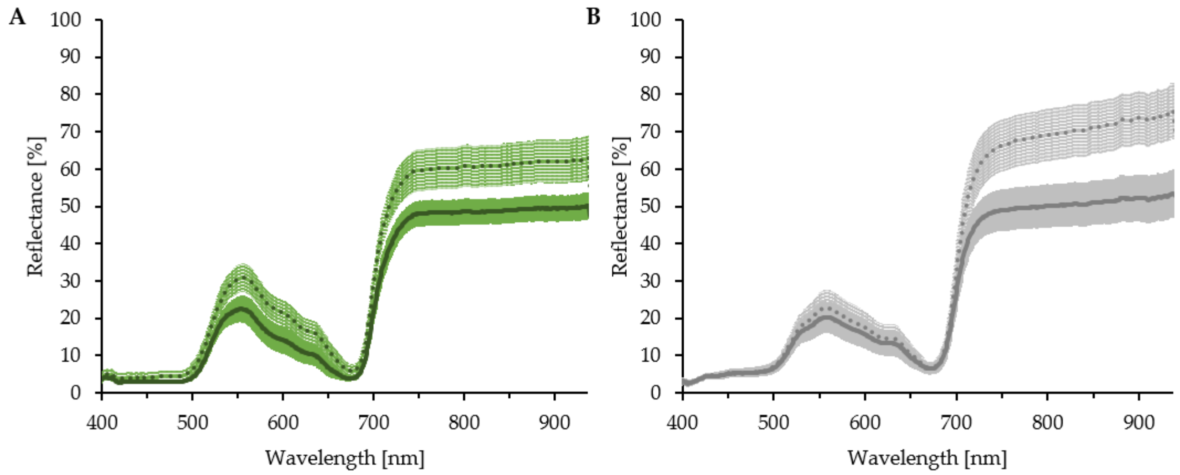

Figure 4. Averaged raw spectra ± SD (n = 40) of *H. macrophylla* subsp. *serrata* ‘Oamacha’ samples measured under different conditions with Red Tide spectrometer in 2019. Green coloring indicates fresh leaves (**A**), grey coloring indicates dried leaves (**B**). Continuous lines represent a black measuring background while hatched lines represent a white measuring background.

Linear regression for ‘Oamacha’ samples is depicted in Figure 5. The training data were reproduced most accurately, for validation samples the prediction did not appear satisfactory. R_p^2 was 0.253 and 0.935 for HG and PD, respectively. As no differences between the measurement conditions could be observed from the model’s discriminant analysis was conducted. Measurement conditions seem to not have an important impact on model predictions. For ‘Oamacha’, the discriminant analysis on black and white measurement backgrounds showed that 62% of HG model predictions and 50% of PD model predictions were grouped correctly. For the leaf water status, fresh or dried leaves, 54% of HG predictions but 75% of PD predictions were performed correctly. Based on this data, only leaf water status seems relevant in PD modeling. Contrary, for 'Amagi Amacha', 46% of the fresh and dried leaves were grouped correctly, while 73% of the predicted PD values were classified correctly in terms of the background color. For 'Odoriko Amacha', 69% and 54% of PD predictions were classified correctly with regard to the measurement background and leaf water status, respectively.

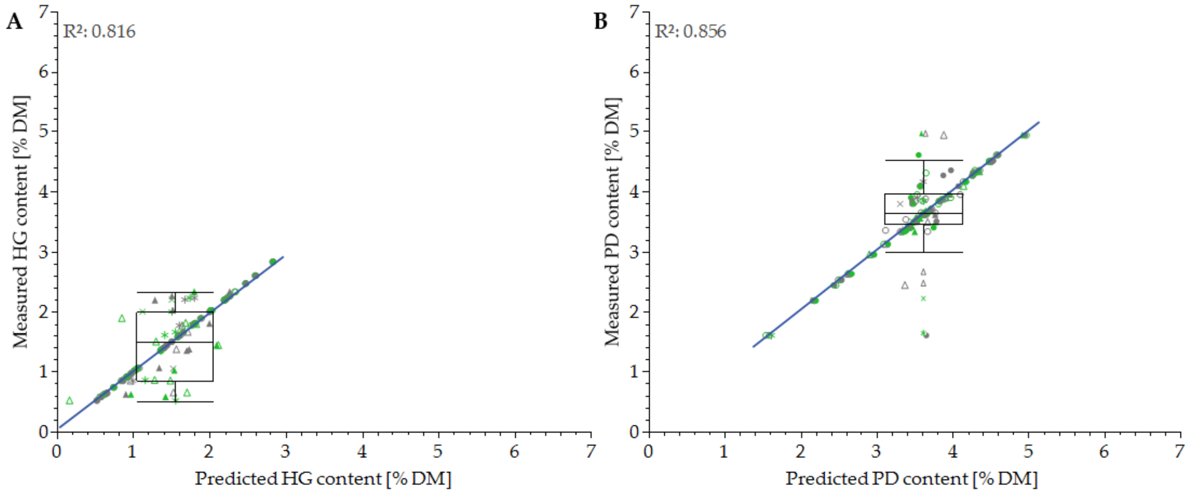

Figure 5. Regression of predicted and measured (Red Tide, 2019) contents of HG (**A**) and PD (**B**) for *H. macrophylla* subsp. *serrata* 'Oamacha' including coefficient of determination. Different symbols indicate the training (o), validation (Δ) and test set (*/ˣ) (HG: training (n = 119), validation (n = 28), test (n = 13); PD: training (n = 118), validation (n = 26), test (n = 16). Green coloring indicates fresh leaves, grey coloring indicates dried leaves. Filled symbols represent a black measuring background (test set: *), not filled symbols represent a white measuring background (test set: ˣ). Boxplots illustrate the variation of the measured DHC contents among the validation- and test set.

3.6 *Effect of spectrometer*

After the differentiation of cultivars appeared to be feasible and measurement conditions did not seem particularly influential on modeling, the following experiment (2021) continued with a larger data set of in-field plants of 'Oamacha' using fresh leaf samples and a black measurement background. The aim was to generate models with reflectance spectra recorded by three different spectrometers and to gain a deeper understanding of wavelengths relevant for PLSR. HG and PD showed great consistency concerning the VIP wavelengths of the reflectance recorded by the Ocean Insight devices. The range of 530–599 nm and between 697 and 737 nm within the VIS spectrum and the ranges of 940–1470 nm and 1497–1664 nm within the NIR spectrum were relevant for both compounds. VIP wavelengths relevant only to HG were

400 nm, 115–116 nm and 616–644 nm. The range between 1470–1497 nm was only relevant to PD (Figure 6).

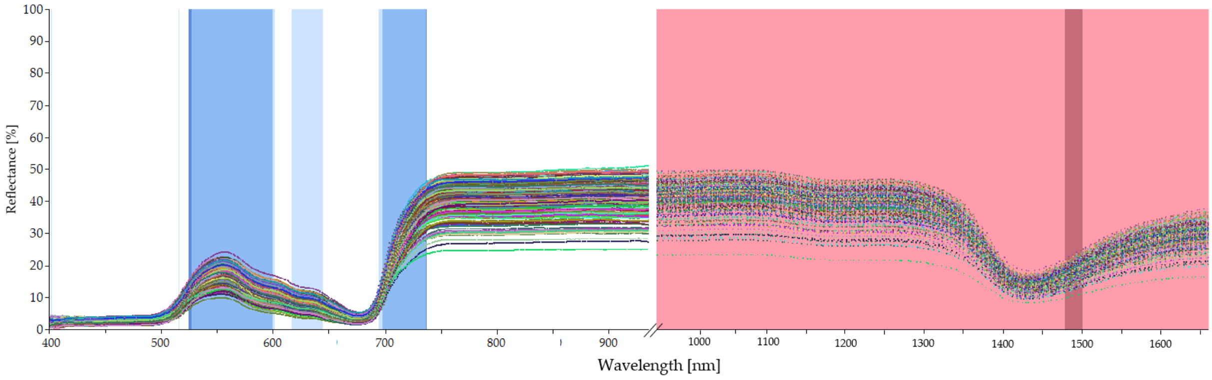

Figure 6. Individual raw spectra of 'Oamacha' samples (n = 296) measured in 2021 with Ocean Insight spectrometers. Colored ranges indicate VIP wavelengths using Red Tide (blue) and Flame-NIR (red). Depending on the DHC compound, ranges have different shading (HG: light shade, PD: dark shade, DHC (HG + PD): medium shade).

With external validation, these VIPs could be confirmed for the most part, and in some cases, variables were added or removed. From here on, models will be named according to the spectrometer used for data acquisition ("Red Tide" and "Flame-NIR").

In the following sample set, DHC contents were spread across a small range, so that a zoomed-in plot of the linear regressions was required. Predictions based on measurements using the two Ocean Insight spectrometers (Red Tide and Flame-NIR, Figure 7 and Figure 8) showed a strong relationship with the reference analytics of the training data. This was also evident from the statistics of the overall model, as the training sets accounted for a large proportion (70%) of the samples. R_{total}^2 (training + validation + test set) was between 0.75 and 0.80 for the two DHCs. The performance of the validation and test sets, on the other hand, was poor, ranging between 0.006 and 0.236 for R_p^2. Contents close to the average value were predicted more accurately, while contents differing from this scattered far outside the regression line. The comparison of R_p^2 revealed that HG contents were modeled slightly more accurately than PD. Additionally, "Flame-NIR" resulted in higher R_p^2 than "Red Tide". More precisely, "Red Tide" yielded $R_p^2 = 0.105$ with a RMSEP of 0.121 and $R_p^2 = 0.006$ with a RMSEP of 0.274 for HG and PD, respectively. "Flame-NIR" resulted in $R_p^2 = 0.236$ with RMSEP of 0.127 for HG and $R_p^2 = 0.115$ with RMSEP of 0.230 for PD. Modeling with measurements from both Ocean Insight spectrometers at the same time (Red Tide + Flame-NIR) resulted in $R_p = 0.118$ for HG. A slightly higher value of $R_p = 0.230$ was determined for PD. It should be noted that the measured contents showed only little variation, which could have made modeling difficult. Nevertheless, the laboratory DHC contents of the validation and test samples within the 25th to 75th percentiles (box shown) were estimated with a precision of ± 0.1% (HG) and ± 0.2% (PD) of the dry matter. However, the exact determination of HG and PD on a leaf-level did not seem possible.

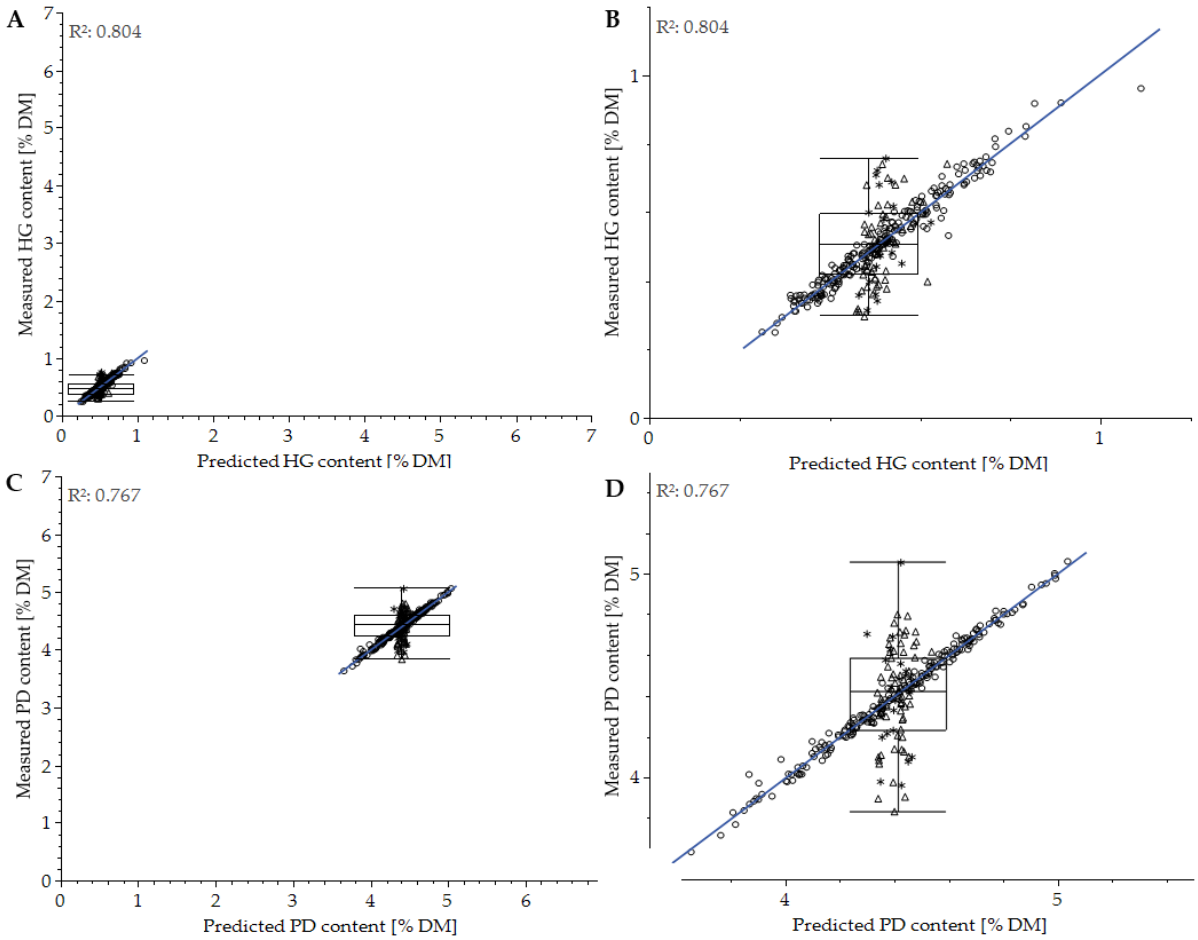

Figure 7. Regression of predicted and measured (Red Tide, 2021) contents of HG (**A**, **B**) and PD (**C**, **D**) for *H. macrophylla* subsp. *serrata* 'Oamacha' including coefficient of determination and a zoomed-in version (**B**, **D**). Different symbols indicate the training (o), validation (Δ), and test set (*) (HG: training (n = 221), validation (n = 50), test (n = 25); PD training (n = 211), validation (n = 64), test (n = 21)). Boxplots illustrate the variation of the measured DHC contents among the validation- and test set.

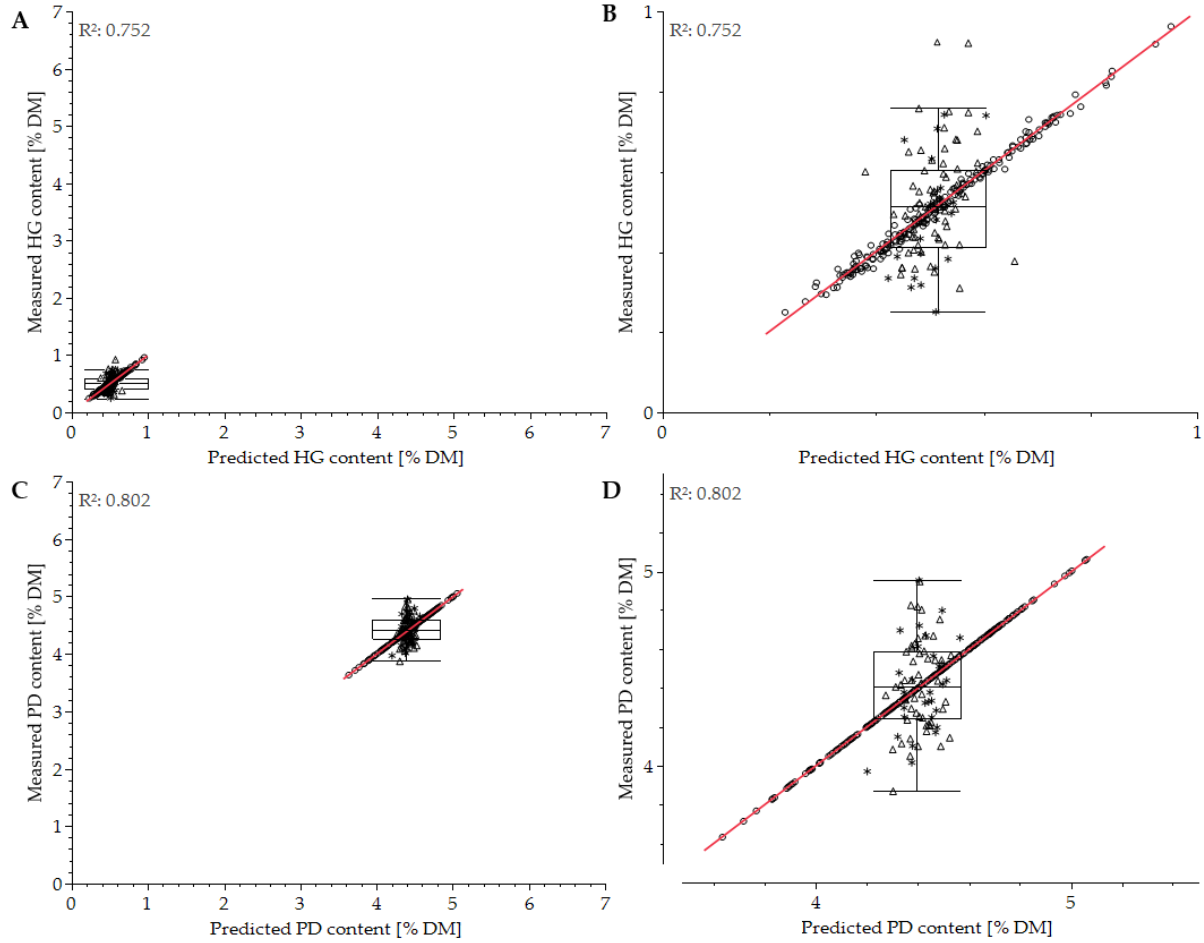

Figure 8. Regression of predicted and measured (Flame-NIR, 2021) contents of HG (**A**, **B**) and PD (**C**, **D**) for *H. macrophylla* subsp. *serrata* 'Oamacha' including coefficient of determination and a zoomed-in version (**B**, **D**). Different symbols indicate the training (o), validation (Δ), and test (*) set (HG: training (n = 215), validation (n = 57), test (n = 24); PD: training (n = 215), validation (n = 53), test (n = 28)). Boxplots illustrate the variation of the measured DHC contents among the validation- and test set.

3.4 *Use of handheld PolyPen RP400*

To simulate measurements on the part of a farmer, a handheld device was used as a third device. By using the PolyPen measurements, VIPs could be reduced to a few, but modeling with just these variables was only possible within a specific division of the samples into the three sets (training, validation and testing) and did not provide reproducible results. Linear regressions of the reference analytics and modeled values are depicted in Figure 9. For the dataset in 2021, "PolyPen" yielded the highest R^2 values for both R_{total}^2 and R_p^2. Modeling of HG resulted in $R_{total}^2 = 0.889$ with a $RMSE_{total}$ of 0.049 and $R_p^2 = 0.422$ with a RMSEP of 0.096. As with Ocean Insight spectrometers, HG contents of the validation and test samples could be determined with an accuracy of nearly ± 0.1% within the interquartile range (25th to 75th percentile). For predicted and measured PD values R_{total}^2 was 0.904 with a $RMSE_{total}$ of 0.084 and $R_p^2 = 0.582$ with a RMSEP of 0.104 showing the most precise result in 2021. For PD, the prediction accuracy of the validation and test samples within the interquartile range was increased to ± 0.1% of the dry matter content. Although the test set consisted of only of five samples, the minimum and maximum content were included in this, showing a representative random sample. Despite, the actual spread of the contents (min. to max. = 1.32%) was reduced by the prediction explaining only 0.216% of the variability.

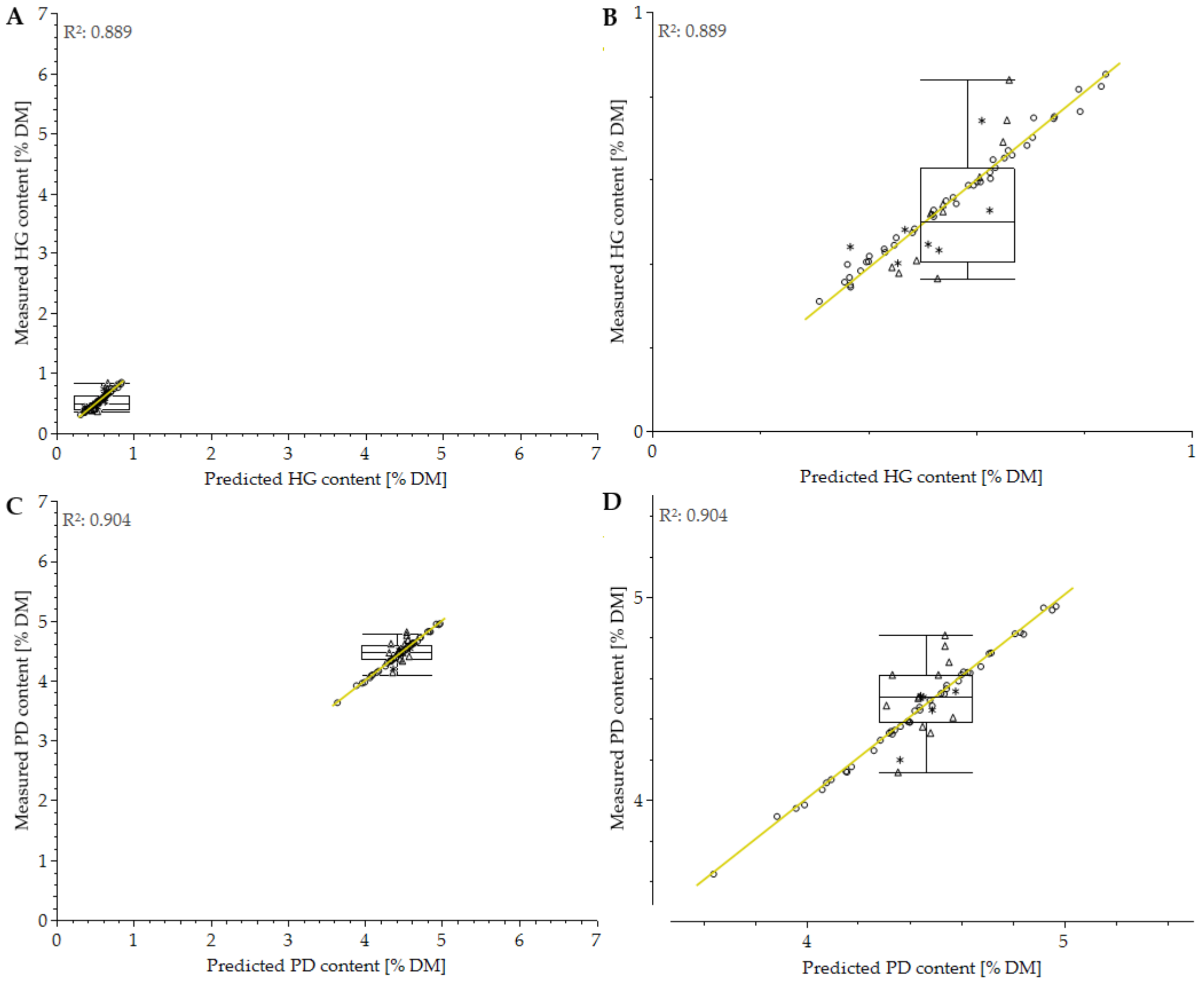

Figure 9. Regression of predicted and measured (PolyPen, 2021) contents of HG (**A**, **B**) and PD (**C**, **D**) for H. macrophylla subsp. serrata 'Oamacha' including coefficient of determination and a zoomed-in version (**B** and **D**). Different symbols indicate the training (o), validation (Δ), and test (*) set (HG: training (n = 42), validation (n = 11), test (n = 7); PD: training (n = 43), validation (n = 12), test (n = 5)). Boxplots illustrate the variation of the measured DHC contents among the validation- and test set.

To determine whether significantly better models could be generated using the PolyPen, R_c^2, R_v^2 und R_p^2 as well as RMSEC, RMSEV and RMSEP of the three spectrometers were compared. In addition, the influence of the proportion of the training set on both parameters was investigated. An overview of the results for the coefficient of regression is shown in Figure 10. For R_p (test set), significant differences were found between the PolyPen and the Ocean Insight spectrometers with a training data proportion of ≥ 70%. Mean values for R_p^2 of the PolyPen models were comparatively significantly higher than those of the Red Tide and Flame-NIR models, namely 0.624 compared to 0.033 and 0.107. Moreover, different ratios of the sample sets influenced the coefficient of determination R_p. The higher the proportion of training data, the higher the R_p^2. It was also shown that the variance of R^2 increased with an increasing proportion of the training data, but was higher overall for the PolyPen. RMSE (Figure not shown) had a lower variance overall, which also tended to increase as the proportion of training increased. As with R_p^2, a significant difference between the handheld device and the other spectrometers was observed for the test set (RMSEP) when using a training proportion of 70%. RMSEP was lower for the PolyPen. At the same time, modeling with measurements from the handheld device proved to be more error-prone. Out of 25 PLSR-trials, with the samples divided into 70% training, 20% validation and 10% test, a model was successfully built 13 times, compared to 23 models with the Ocean Insight devices.

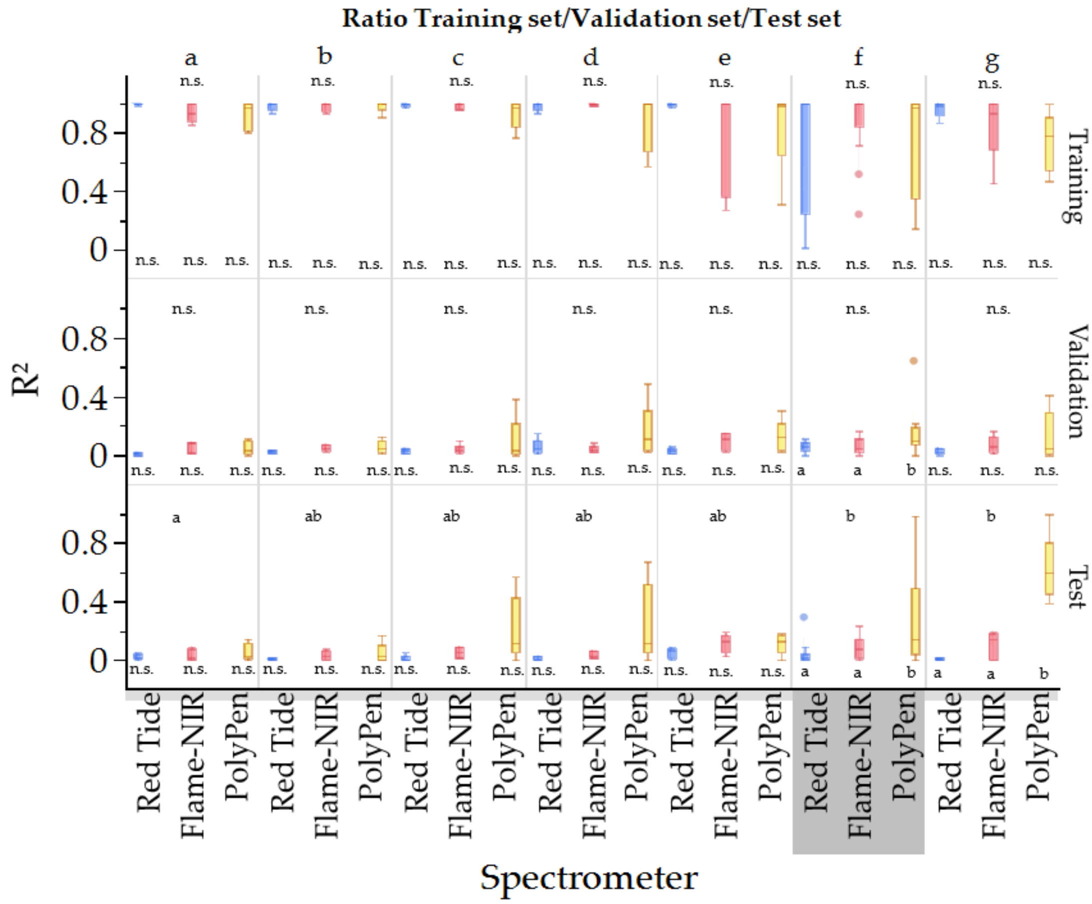

Figure 10. Coefficient of determination (R^2) for PLSR models of phyllodulcin (PD) developed using measurements of *H. macrophylla* subsp. *serrata* samples in 2021. Different coloring indicates the spectrometers used (blue: Red Tide, red: Flame-NIR, yellow: PolyPen). Letters a-g represent different splitting of the sample set (ratio Training/Validation/test set: a = 2/5.3/2.7; b = 3/4.7/2.3; c = 4/4/2; d = 5/3.3/1.7; e = 6/2.7/1.3; f = 7/2/1; g = 8/1.3/0.7). N = 5 except letter f (ratio used for modeling): Red Tide n = 23; Flame-NIR n = 23; PolyPen n = 13. Significant differences were determined by ANOVA and Tuckey-HSD as post-hoc procedure (p = 0.05) and are indicated by letters (a, ab, b). Letters above the boxplots refer to differences between the ratios (row), letters below the boxplots refer to differences between the spectrometers within a ratio (box).

As models for a single cultivar did not allow conclusions to be drawn about the exact content of individual leaves, mean contents of the lab analytics and the predicted values (test set) were used for comparison. As shown in Figure 11, box plots of the reference analytics from 2021 were well represented by the modeled DHC contents. For both HG and PD, the measured and predicted values agreed to within one decimal place. For HG, differences of 0.002% (Red Tide), 0.012% (Flame-NIR) and 0.038% (PolyPen) of the dry matter content were determined. Modeled PD contents deviated < 1% from the reference analytics, resulting in differences of 0.004% (Red Tide), 0.007% (Flame-NIR) and 0.017% (PolyPen) in the dry matter content.

Consequently, the PLSR models established appeared suitable to adequately reproduce an average value of sampled in-field plants. Greater discrepancies were found for the models "measurement conditions" and "cultivar differentiation". When using the "measurement conditions" data, the mean HG content of 1.514% was well modeled (1.472%), whereas the values for PD were on average 0.234% lower than lab contents. The highest variance (0.332%) was observed when comparing the mean values of PD of "cultivar differentiation", which was due to the unequal distribution of cultivars into training and test samples.

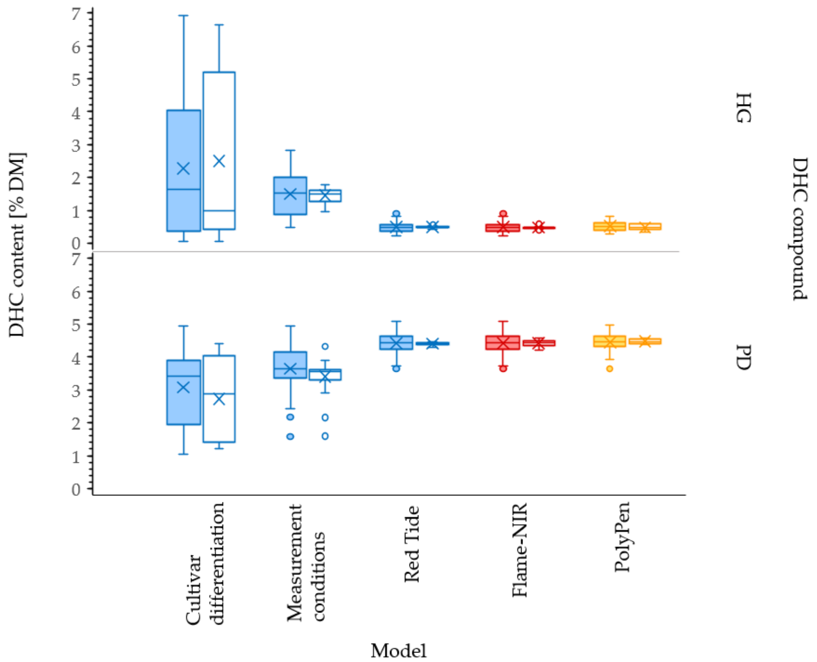

Figure 11. DHC (HG: hydrangenol; PD: phyllodulcin) contents from chemical analysis via UPLC (filled box) and modeled contents (test set, colorless box) for different models computed and compilated in this study based on the five models shown in this study.

Table 2. Statistical evaluation of the goodness of fit modeling hydrangenol (HG) and phyllodulcin (PD) including coefficient of determination (R^2) and Root Mean Square Error (RMSE) for the calibration- (R_c^2/RMSEC), validation- (R_v^2/RMSEV) and prediction (test) sets (R_p^2/RMSEP) and the overall model (R_{total}^2/$RMSE_{total}$) consisting of all three sets.

Model	DHC	Calibration		Validation		Prediction		Overall model	
		R_c^2	RMSEC	R_v^2	RMSEV	R_p^2	RMSEP	R_{total}^2	$RMSE_{total}$
Cultivar differentiation	HG	0.919	0.569	0.998	0.099	0.998	0.116	0.941	0.496
	PD	0.910	0.340	0.893	0.344	0.910	0.340	0.921	0.305
Measurement conditions	HG	1.000	0.007	0.239	0.523	0.253	0.549	0.816	0.273
	PD	0.861	0.261	0.762	0.297	0.935	0.196	0.856	0.261
Red Tide 2021	HG	0.959	0.028	0.276	0.101	0.105	0.121	0.804	0.059
	PD	0.989	0.029	0.114	0.221	0.006	0.274	0.767	0.128
Flame-NIR 2021	HG	0.989	0.014	0.087	0.124	0.236	0.127	0.752	0.066
	PD	1.000	0.001	0.024	0.229	0.115	0.230	0.802	0.118
Red Tide + Flame-NIR	HG	1.000	0.001	0.173	0.129	0.118	0.134	0.753	0.066
	PD	0.998	0.012	0.076	0.219	0.230	0.225	0.820	0.112
PolyPen 2021	HG	0.991	0.015	0.863	0.062	0.422	0.096	0.889	0.049
	PD	0.998	0.015	0.194	0.182	0.582	0.104	0.904	0.084

4. Discussion

This experiment investigated the possibilities of hyperspectral modeling for in-field applications. Therefore, known difficulties and restrictions of hyperspectral measurements of field conditions in comparison to laboratory conditions (Udelhoven et al., 2001) were purposefully taken into account as well. This led to a simulation of practicability for rapid in-field measurements in combination with model quality for HG and PD. For this purpose, the accurate depiction of mean HG and PD contents via hyperspectral modeling was sufficient.

The UPLC analysis of DHC contents revealed that the three cultivars show distinct patterns of HG and PD contents. ‘Odoriko Amacha’ yielded the highest HG contents and lowest amounts of PD. ‘Amagi Amacha’ expressed the contrary pattern with high contents of PD while yielding the lowest contents of HG. Tea-hortensia cultivar ‘Oamacha’ was the middle-ground in regard of HG and yielded PD contents that were at the same level as the ones of ‘Amagi Amacha’. A similar pattern for these three cultivars was previously found with one key difference as ‘Amagi Amacha’ yielded significantly higher PD contents than ‘Oamacha’ (Moll et al., 2021b). This overlap might be due to a known pattern of seasonal changes (Ujihara et al., 1995).

Additionally, this pattern of seasonal changes is more pronounced for 'Amagi Amacha' than for other cultivars (unpublished data). This overlap is one major factor influencing the cultivar differentiation via hyperspectral modeling. Besides HG and PD contents, morphological and physiological differences of the three cultivars could lead to the possible differentiation of cultivars. Leaf hairs (Ge et al., 2012) and leaf wax (Lu, 2013) influence the reflectance and therefore could allow for the distinction of cultivars. Additionally, adaxial and abaxial surface influence hyperspectral modeling for chlorophyll (Lu and Lu, 2015) while nitrogen and chlorophyll (Yamashita et al., 2020), as well as general nutrient status (Gong et al., 2002) are common parameters to be modeled. During the interpretation of such VIS-NIR models, two parameters have to be taken into consideration besides plant morphology, accuracy and representativity of measurements. UPLC generally performs at a reasonably well accuracy (Saviano et al., 2015), but as the dynamics of hydrangenol and phyllodulcin in leaves of tea-hortensias are not clear yet, hyperspectral measurements might weaken model quality. This could be due to a lack of representativity of point-measurements on the leaves or an uneven distribution of dihydroisocoumarins in the leaf.

As the contents of HG and PD in relation to the respective cultivars performed similarly well for measured and modeled contents, the modeling of HG and PD for the purpose of cultivar differentiation was successful. The results also show that accurate determinations of HG and PD are not possible from the models developed in this study, while the best results were obtained with the hand-held spectrometer (Table 2). Other spectrometers detecting in the UV and short wavelength infrared (SWIR) regions have previously been investigated for the detection of phenolic compounds and reached $R^2 = 0.91$ for UV and $R^2 = 0.99$ for SWIR (Tschannerl et al., 2019). Based on these results, different spectrometers that cover other spectral ranges or spectrometers that cover smaller ranges but yield a higher resolution might lead to more accurate models.

The VIP wavelengths for HG and PD overlap to a large extent. Still, key wavelengths that only yield information for HG or PD were identified. This leads to the assumption that further research in the field of HG and PD modeling could yield more precise models based on lesser wavelengths. The models shown in this study show two important features. On the first sight, the model quality does not allow for an approximation of HG and PD in single plants for tea-hortensia cultivar 'Oamacha'. The regression clearly indicates R^2 values that show that no relation of measured and modeled constituent contents was achieved. Consequently, the models are not yet feasible to replace the well-established UPLC analysis. The major feature of such models in this study was to give farmers a decision support. The comparison of mean HG and

PD values via UPLC and the models shown in this study reveal that the Red-Tide modeled mean HG and PD highly accurate in the test-set. Hence, the purpose of the models in this study was successfully achieved as Red-Tide models provide a clear overview of HG and PD contents in the field overall. As single plants are not of interest for farmers, this overview is the key feature necessary for harvest date decisions. The quality of models might be negatively influenced by heterogeneity of plant material as well. This phenomenon would be ever-present for such applications and is therefore considered for HG and PD models for farmers applications.

The results of this study indicate that it is possible to differentiate tea-hortensia cultivars via hyperspectral modeling. Parallel, the approximation of mean HG and PD contents were successful as well. Based on these results further research regarding in-field measurements with more cultivars under different environments and stages during the production cycle are needed. Also, the implementation of spectrometers covering different wavelengths could improve model quality. This would enable more precise applications for research surrounding tea-hortensias. Especially breeding and the faster selection based on HG and PD contents would benefit from accurate models. For the in-field cultivation the incorporation of UAVs presents further improvements, as the detection of wavelengths for multiple plants could be performed in one flight.

5. Conclusions

Dihydroisocoumarin (DHC) contents of hydrangenol (HG) and phyllodulcin (PD) in tea-hortensias have been shown to express seasonal changes (Ujihara et al., 1995) as well as cultivar specific concentrations and reactions to the environment (Moll et al., 2021b). Chemical analysis of HG and PD are no sufficient option for farmers to determine optimal harvest dates. Three different spectrometers were investigated for their suitability to model HG and PD contents in three different tea-hortensia cultivars (‘Amagi Amacha’, ‘Oamacha’, and ‘Odoriko Amacha’). Special focus was put on feasibility and robustness of spectrometer setups for a farm-based application. The differentiation of cultivars based on model predictions showed to be as accurate as predictions based on actual HG and PD contents while the effect of measurement background as well as leaf water status (fresh or dried) seems neglectable for such applications. Model quality for all three spectrometers tested did not provide sufficient accuracy to predict HG and PD for lab applications. Still, the models allow for an estimation of mean HG and PD contents on a field scale to give farmers an indication for higher or lower DHC contents to support decision-making in regard to harvest dates.

Chapter 5 – General Conclusions

The main objective of this work was to investigate the possibilities of quality assessment for tea-hortensias via non-invasive phenotyping. This was performed with the use of tea-hortensias as feedstock for phyllodulcin and their optimized cultivation with regard to shading. For this, three *H. macrophylla* subsp. *serrata* cultivars (‘Amagi Amacha’, ‘Oamacha’, and ‘Odoriko Amacha’) as well as a segregating F_1 breeding population from a cross of ‘Odoriko Amacha’ and ‘Yae Amacha’ were investigated.

The results described in chapter 2 revealed that, in contrast to the cultivation of *H. macrophylla* as an ornamental flowering plant, shading seems unnecessary for a successful cultivation of tea-hortensias as an industrial plant to gain high biomass accumulation and PD content. Still, the three cultivars showed different accumulation of HG and PD as well as different physiological reactions towards sun exposure. This leads to the conclusion that HG and PD may not be influenced by sun exposure and the different microclimates under different shading regimes. In conclusion, the hypothesis of the necessity of shading for tea-hortensia cultivation can be rejected. The second hypothesis is confirmed, as the experiment also leads to the conclusion that different tea-hortensia cultivars show different reaction towards sun exposure. Additionally, the experiment revealed that HG and PD contents are primarily predetermined by genetics and external stressor only

Chapter 3 builds on the results described in chapter 2 to identify the interaction of environment and PD content as well as the conversion rates of HG into PD. This was performed with the three cultivars used in chapter 2 while adding the F_1 breeding population as a further pool of information. Data from 12 distinct sub-experiments (SEs) was used to investigate HG and PD contents under different environmental and artificial stressors. Observations build on the chemometric Difference Phyllodulcin Index (cNDPI) revealed that while absolute contents of dihydroisocoumarins underlie seasonal changes, the conversion rate is highly stable for each cultivar. This was also successfully shown for the breeding population (F_1 from ‘Odoriko Amacha’ x ‘Yae Amacha’) in three distinct environments. Additionally, *H. macrophylla* subsp. *serrata* ‘Amagi Amacha’, ‘Oamacha’, and ‘Odoriko Amacha’ were investigated over the course of the 12 SEs to determine the reliability of the cNDPI and therefore the information of the underlying biological information. A significant differentiation of cultivars is possible, with low rates of misclassification. This finding based on the cNDPI investigations confirms the third hypothesis that the conversion of HG into PD is cultivar dependent and can be used as a chemometric marker. Additionally, the investigations of cNDPI confirmed the idea that contents of PD are highly stable towards external effects (while being affected by seasonal fluctuations) and are mainly genetically determined. Furthermore, conversion of HG into PD is stable for the cultivars as well as the breeding population. Based on this information, cNDPI is a suited proxy in plant breeding, especially when combined with QTL analytics. Therefore, the fourth hypothesis regarding feasibility and reliability of cNDPI for plant breeding can be confirmed as well. This enables for a new perspective in targeted plant breeding for high performance tea-hortensias when inbreeding *H. macrophylla* for their robust growth and larger leaves. Still, the quantifications of HG and PD for the preceding trials had to be performed in a lab. Especially in plant breeding large numbers of plants would have to be screened to find the "best" plant to develop further.

Chapter 4 tried to find a solution to the arising problem from chapters 2 and 3: the number of chemical analyses needed for clear results in cultivar optimization and plant selection for plant breeding. Therefore, the possibility of hyperspectral modeling of HG and PD was investigated. While it was possible to set up a model that enables farmers and growers of tea-hortensias to identify optimal harvest dates, the accuracy was not yet sufficient to combine hyperspectral modeling and cNDPI to allow for a non-invasive determination of cNDPI. Additionally, the effect of measurement conditions showed to have an impact. Therefore, the fifth hypothesis of model accuracy being influenced by measurement conditions is to be accepted. Hypothesis six regarding suitability of VIS-NIR spectroscopy for modeling is either rejected or accepted depending on application purposes. Highly accurate and reliable measurements were not possible, while measurements as a proxy for the field level were successfully achieved.

The results presented in this work reveal several fields of research. First, optimization of hyperspectral model quality is needed to make cNDPI calculations faster, as no lab analyses would be needed. This would enable plant breeders in the field of tea-hortensias to use such models in the selection process by quantifying PD contents as well as conversion efficiency. The experiments compiled in this work did not show any influence of external effects on PD contents. Still, further research is needed to possibly find factors to increase PD synthesis and yield. As shown in chapter 1.2.2, flowering showed to decrease biomass production in *Hydrangea*. Therefore, flower-inhibition is needed for optimal yield. Additionally, targeted nutrient application and fertilization management could increase biomass production and therefore total PD yield which was not investigated here. Based on the results in this work, the cultivation of tea-hortensias in Europe on an industrial scale seems non-problematic. But not only cultivation and quality-assessment are to be considered when putting a holistic view on tea-hortensias as feedstock for PD. The chain of synthesis of HG and PD is not fully understood yet and therefore potential points of optimization remain unclear. Consumer acceptance showed to be a highly discussed topic in regard to *Stevia rebaudiana*, as discussed in chapter 1. The same topic needs to be addressed for phyllodulcin from tea-hortensia as well.

Finally, this thesis was the first attempt to combine different types of quality assessment of tea-hortensias. Chemical analysis and a new chemometric index (cNDPI) were used for DHC content and conversion efficiency of HG into PD. Vegetation indices and modeling of complex hyperspectral analyses were used to develop feasible and reliable proxies to predict HG and PD contents in tea-hortensias. The establishment of such quality assessment tools opens the way for the successful transformation of an ornamental plant with a traditional usage (tea-hortensia), into an industrial crop to produce bio-based high value products (hydrangenol, phyllodulcin). The combination of the CNDPI and hyperspectral modeling could enable breeders in their efforts for breeding and selecting a robust and high-yielding tea-hortensia. Additionally, the implementation of vegetation indices and hyperspectral modeling combined with traditional agronomic know-how would enable farmers and producers of tea-hortensias to establish not only a successful cultivation but at the same time monitor tea-hortensia quality. The results presented in this work build the foundation of optimization and quality assessment of the renewable resource “tea-hortensia” as feedstock for dihydroisocoumarins.

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References

Acosta-Estrada, B.A., Gutiérrez-Uribe, J.A., Serna-Saldívar, S.O., 2014. Bound phenolics in foods, a review. Food Chem. 152, 46–55.

Aguilera, A.M., Aguilera-Morillo, M.C., 2013. Comparative study of different B-spline approaches for functional data. Math. Comput. Model. 58, 1568–1579.

Aguilera-Morillo, M.C., Aguilera, A.M., 2015. P-spline Estimation of Functional Classification Methods for Improving the Quality in the Food Industry. Commun. Stat. B: Simul. Comput. 44, 2513–2534.

Aleixandre-Tudo, J.L., Nieuwoudt, H., Aleixandre, J.L., Du Toit, W.J., 2015. Robust Ultraviolet-Visible (UV-Vis) Partial Least-Squares (PLS) Models for Tannin Quantification in Red Wine. J. Agric. Food Chem. 63, 1088–1098.

Al-Tamimi, N., Brien, C., Oakey, H., Berger, B., Saade, S., Ho, Y.S., Schmöckel, S.M., Tester, M., Negrão, S., 2016. Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. Nat. Commun. 7, 13342.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems. Agric. Ecosys. Environ. 74, 19–31.

Anderson, N., Weiland, J., Pharis, J., Gagné, W., Janiga, E., Rosenow, M.J., 2009. Comparative forcing of Hydrangea macrophylla ‘Bailer’ as a florist's hydrangea. Sci. Hortic. 122, 221–226.

Anderson, N.T., Walsh, K.B., Wulfsohn, D., 2021. Technologies for Forecasting Tree Fruit Load and Harvest Timing—From Ground, Sky and Time. Agronomy 11, 1409.

Angert, A., Biraud, S., Bonfils, C., Henning, C., Buermann, W., Pinzon, J., Tucker, C.J., Fung, I., 2005. Drier summers cancel out the CO2 uptake enhancement induced by warmer springs. PNAS 102, 10823–10827.

Artetxe, A., Terés, V., Beunza, A.I., 1997. Effects of Container Size and substrates on Hydrangea macrophylla growth. Proc. Int'l Symp. on Growing Media and Plant Nutrition - Ed. R.U. Roeber, 419–424.

Asen, S., Cathey, H.M., Stuart, N.W., 1960. Enhancement of Gibberellin growth-promotiong Activity by Hydrangenol Isolated From Leaves of Hydrangea macrophylla. Plant Physiol. 35, 816–819.

Asgari, N., Ayoubi, S., Demattê, J.A.M., Dotto, A.C., 2020. Carbonates and organic matter in soils characterized by reflected energy from 350–25000 nm wavelength. J. Mt. Sci. 17, 1636–1651.

Balasundram, N., Sundram, K., Samman, S., 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99, 191–203.

Bannari, A., Morin, D., Bonn, F., Huete, A.R., 1995. A review of vegetation indices. Remote Sens. Rev. 13, 95–120.

Barak, P., 1995. Smoothing and Differentiation by an Adaptive-Degree Polynomial Filter. Anal. Chem. 67, 2758–2762.

Barbin, D.F., ElMasry, G., Sun, D.-W., Allen, P., 2012. Predicting quality and sensory attributes of pork using near-infrared hyperspectral imaging. Anal. Chim. Acta 719, 30–42.

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environ. Exp. Bot. 32, 85–100.

Barnes, R.J., Dhanoa, M.S., Lister, S.J., 1989. Standard Normal Variate Transformation and De-Trending of Near-Infrared Diffuse Reflectance Spectra. Appl. Spectrosc. 43, 772–777.

Barry, R.D., 1964. Isocoumarins. Developments Since 1950. Chem. Rev. 64, 229–260.
Basker, D., Negbi, M., 1983. Uses of saffron. Econ. Bot. 37, 228–236.
Bawane Adesh, A., Gopalakrishna, B., Akki Kusum, S., Tiwari, O., 2012. An Overvire on Stevia: A Natural Calorie Free Sweetener. IJAPBC 1, 362–368.
Baydar, H., Baydar, N.G., 2005. The effects of harvest date, fermentation duration and Tween 20 treatment on essential oil content and composition of industrial oil rose (Rosa damascena Mill.). Ind. Crops Prod. 21, 251–255.
Baysal-Gurel, F., Kabir, M.N., Blalock, A., 2016. Foliar diseases of Hydrangea. Tennessee State University Fact Sheet ANR-PATH-5-2016.
Bellisle, F., 1999. Glutamate and the UMAMI taste: sensory, metabolic, nutritional and behavioural considerations. A review of the literature published in the last 10 years. Neurosci. Biobehav. Rev. 23, 423–438.
Bernáth, J., 2002. Strategies and Recent Achievements in Selection of Medicinal and Aromatic Plants. Acta Hortic., 115–128.
Bertoli, A., Sárosi, S., Bernáth, J., Pistelli, L., 2010. Characterization of Some Italian Ornamental Thyme by Their Aroma. Nat. Prod Commun. 5, 1934578X1000500.
Bi, G., Scagel, C.F., Harkess, R., 2008. Rate of nitrogen fertigation during vegetativegrowthand spray applications of urea in the fall altersgrowthand flowering of florists' hydrangeas. HortScience 43, 472–477.
Blüthgen, N., Klein, A.-M., 2011. Functional complementarity and specialisation: The role of biodiversity in plant–pollinator interactions. Basic Appl. Ecol. 12, 282–291.
Bogers, R.J., Craker, L.E., Lange, D. (Eds.), 2006. Medicinal and Aromatic Plants. Springer Netherlands, Dordrecht.
Boochs, F., Kupfer, G., Dockter, K., Kühbauch, W., 1990. Shape of the red edge as vitality indicator for plants. Int. J. Remote Sens. 11, 1741–1753.
Bordi, P.L., Palchak, T., Verruma-Bernardi, M.R., Cho, H.C., 2016. Adult Acceptance of Chocolate Milk Sweetened with Stevia. J. Culin. Sci. Technol. 14, 216–221.
Braca, A., Bader, A., Tommasi, N. de, 2012. Plant and Fungi 3,4-Dihydroisocoumarins. In: Studies in Natural Products Chemistry Volume 37, vol. 37. Elsevier, pp. 191–215.
Brugger, A., Behmann, J., Paulus, S., Luigs, H.-G., Kuska, M.T., Schramowski, P., Kersting, K., Steiner, U., Mahlein, A.-K., 2019. Extending Hyperspectral Imaging for Plant Phenotyping to the UV-Range. Remote Sens. 11, 1401.
Cardone, L., Castronuovo, D., Perniola, M., Cicco, N., Candido, V., 2020. Saffron (Crocus sativus L.), the king of spices: An overview. Sci. Hortic. 272, 109560.
Castellano, S., Scarascia Mugnozza, G., Russo, G., Briassoulis, D., Mistriotis, A., Hemming, S., Waaijenberg, D., 2008. Plastic Nets in Agriculture: A General Review of Types and Applications. App. Eng. Agric. 24, 799–808.
Castro, E.M. de, Pinto, J., Bertolucci, S., Malta, M.R., Cardoso, M.d., Silva, F.A. de, 2006. Coumarin Contents in Young Mikania glomerata Plants (Guaco) under Different Radiation Levels and Photoperiod. Acta Farm. Donaerense 25, 387–392.
Cen, H., He, Y., 2007. Theory and application of near infrared reflectance spectroscopy in determination of food quality. Trends Food Sci. Technol. 18, 72–83.
Cendrero-Mateo, M.P., Muller, O., Albrecht, H., Burkart, A., Gatzke, S., Janssen, B., Keller, B., Körber, N., Kraska, T., Matsubara, S., Li, J., Müller-Linow, M., Pieruschka, R., Pinto, F., Rischbeck, P., Schickling A., Steier, A., Watt, M., Schurr, U., Rascher, U., 2017. Field Phenotyping: Concepts and Examples to Quantify Dynamic Plant Traits across Scales in the Field. CRC Press, Taylor & Francis, Boca Raton, USA., 53–80.

Céron-Rojas, J.J., Crossa, J., 2018. Linear Selection Indices in Modern Plant Breeding. Springer International Publishing, Cham, 271 pp.
Chalker-Scott, L., 1999. Environmental Significance of Anthocyanins in Plant Stress Responses. Photochem. Photobiol. 70, 1–9.
Chang, C.-W., Laird, D.A., Mausbach, M.J., Hurburgh, C.R., 2001. Near-Infrared Reflectance Spectroscopy-Principal Components Regression Analyses of Soil Properties. Soil Sci. Soc. Am. J. 65, 480–490.
Chattopadhyay, S., Raychaudhuri, U., Chakraborty, R., 2014. Artificial sweeteners - a review. J. Food Sci. Technol. 51, 611–621.
Cheng, W., Sun, D.-W., Cheng, J.-H., 2016. Pork biogenic amine index (BAI) determination based on chemometric analysis of hyperspectral imaging data. LWT - Food Sci. Technol. 73, 13–19.
Cheynier, V., 2012. Phenolic compounds: from plants to foods. Phytochem. Rev. 11, 153–177.
Chiko, A.W., Godkin, S.E., 1986. Occurence of Alfalfa Mosaic, Hydrangea Ringspot, and Tobacco Ringspot Viruses in Hydrangea spp. in British Columbia. Plant Dis. 70, 541-544.
Chiluwal, A., Bheemanahalli, R., Perumal, R., Asebedo, A.R., Bashir, E., Lamsal, A., Sebela, D., Shetty, N.J., Krishna Jagadish, S.V., 2018. Integrated aerial and destructive phenotyping differentiates chilling stress tolerance during early seedling growth in sorghum. Field Crops Res. 227, 1–10.
Christaki, E., Bonos, E., Giannenas, I., Florou-Paneri, P., 2012. Aromatic Plants as a Source of Bioactive Compounds. Agriculture 2, 228–243.
Clark, R.N., Roush, T.L., 1984. Reflectance spectroscopy: Quantitative analysis techniques for remote sensing applications. J. Geophys. Res. 89, 6329–6340.
Clevers, J., Jong, S.M. de, Epema, G.F., van der Meer, F.D., Bakker, W.H., Skidmore, A.K., Scholte, K.H., 2002. Derivation of the red edge index using the MERIS standard band setting. Int. J. Remote Sens. 23, 3169–3184.
Cochran, D.R., Fulcher, A., 2013. Type and Rate of Plant Growth Regulator Influence Vegetative, Floral Growth, and Quality of Little Lime™ Hydrangea. HortTechnology 23, 306–311.
Collins, W., 1978. Remote Sensing of Crop Type and Maturity. Photogramm. Eng. Remote Sens. 44, 43–55.
Condon, M., 2017. Evaluating hydrangea performance. Acta Hortic. 1174, 241–248.
Cozzolino, D., 2009. Near infrared spectroscopy in natural products analysis. Planta Med. 75, 746–756.
Cozzolino, D., 2015. The role of visible and infrared spectroscopy combined with chemometrics to measure phenolic compounds in grape and wine samples. Molecules 20, 726–737.
Davari, N., Jouri, M.H., Ariapour, A., 2011. Comparison of Measurement Indices of Diversity, Richness, Dominance, and Evenness in Rangeland Ecosystem (Case Study: Jvaherdeh-Ramesar). J. Rangel. Sci. 2, 389–398.
Dietrich, S., Valio, I., 1973. Effect of coumarin and its derivatives on the growth of Pythium and other fungi. Trans. Br. Mycol. Soc. 61, 461–469.
Dilshara, M.G., Jayasooriya, R.G.P.T., Lee, S., Jeong, J.B., Seo, Y.T., Choi, Y.H., Jeong, J.-W., Jang, Y.P., Jeong, Y.-K., Kim, G.-Y., 2013. Water extract of processed Hydrangea macrophylla (Thunb.) Ser. leaf attenuates the expression of pro-inflammatory mediators by suppressing Akt-mediated NF-κB activation. Environ. Toxicol. Pharmacol. 35, 311–319.
Dirr, M.A., 2004. Hydrangeas for American Gardens. Timer Press, Portland, OR.

Dougherty, R.F., Quinn, L.D., Endres, A.B., Voigt, T.B., Barney, J.N., 2014. Natural History Survey of the Ornamental Grass Miscanthus sinensis in the Introduced Range. Invasive plant sci. manag. 7, 113–120.
Duke, J.A., 1973. Utilization of Papaver. Econ. Bot. 27, 390–400.
Dunn, W.J., Wold, S., Edlund, U., Hellberg, S., Gasteiger, J., 1984. Multivariate structure-activity relationships between data from a battery of biological tests and an ensemble of structure descriptors: The PLS method. Quant. Struct.-Act. Relat. 3, 131–137.
Eggersdorfer, M., Meyer, J., Eckes, P., 1992. Use of renewable resources for non-food materials. FEMS Microbiol. Rev. 103, 355–364.
Eid, G.M., Albatal, N., Haddad, S., 2016. Effect of Photoperiod on the Flowering of Some Cultivars of Hydrangea (*Hydrangea macrophylla*). Int. J. Hortic. 6, 1–7.
Ercioglu, E., Velioglu, H.M., Boyaci, I.H., 2018a. Chemometric Evaluation of Discrimination of Aromatic Plants by Using NIRS, LIBS. Food Anal. Methods 11, 1656–1667.
Ercioglu, E., Velioglu, H.M., Boyaci, I.H., 2018b. Determination of terpenoid contents of aromatic plants using NIRS. Talanta 178, 716–721.
Famili, A., Shen, W.-M., Weber, R., Simoudis, E., 1997. Data Preprocessing and Intelligent Data Analysis. Intell. Data Anal. 1, 3–23.
Ferrante, A., Mariani, L., 2018. Agronomic Management for Enhancing Plant Tolerance to Abiotic Stresses: High and Low Values of Temperature, Light Intensity, and Relative Humidity. Horticulturae 4, 21.
Filella, I., Peñuelas, J., 1994. The red edge position and shape as indicators of plant chlorophyll content, biomass and hydric status. Int. J. Remote Sens. 15, 1459–1470.
Fiorani, F., Schurr, U., 2013. Future scenarios for plant phenotyping. Annu. Rev. Plant Biol. 64, 267–291.
Fujii, T., Yoshida, T., 2005. The characteristics of amacha varieties in Hydrangea. Japanese J. Crop Sci., 52–57.
Fujita, M., Tanabata, T., Urano, K., Kikuchi, S., Shinozaki, K., 2018. RIPPS: A Plant Phenotyping System for Quantitative Evaluation of Growth Under Controlled Environmental Stress Conditions. Plant Cell Physiol. 59, 2030–2038.
Fulcher, A., Owen, J.S., LeBude, A., 2016. Hydrangea Production: Species-Specific Production Guide. Available online: https://extension.tennessee.edu/publications/Documents/PB1840-B.pdf (accessed on 17. January 2022).
Gamon, J., Field, C.B., Goulden, M.L., Griffin, K.L., Hartley, A.E., Joel, G., Peñuelas, J., Valentini, R., 1995. Relationships Between NDVI, Canopy Structure, and Photosynthesis in Three Californian Vegetation Types. Ecol. Appl. 5, 28–41.
Gamon, J.A., Peñuelas, J., Field, C.B., 1992. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sens. Environ. 41, 35–44.
Garbulsky, M.F., Peñuelas, J., Gamon, J., Inoue, Y., Filella, I., 2011. The photochemical reflectance index (PRI) and the remote sensing of leaf, canopy and ecosystem radiation use efficienciesA review and meta-analysis. Remote Sens. Environ. 115, 281–297.
Ge, H., Lu, S., Zhao, Y., 2012. Effects of Leaf Hair on Leaf Reflectance and Hyperspectral Vegetation Indices. Spectrosc. Spec. Anal. 32, 439–444.
Geladi, P., Kowalski, B.R., 1986. Partial least-squares regression: a tutorial. Anal. Chim. Acta 185, 1–17.
Gernaat, D.E.H.J., Boer, H.S. de, Daioglou, V., Yalew, S.G., Müller, C., van Vuuren, D.P., 2021. Climate change impacts on renewable energy supply. Nat. Clim. Chang. 11, 119–125.

Ghazshazi, H., Miguel, M.G., Hasnaoui, B., Sebei, H., Ksontini, M., Figueiredo, A.C., Pedro, J.G., Barroso, J.G., 2010. Phenols, essential oils and carotenoids of Rosa canina from Tunisia and their antioxidant activities. Afr. J. Biotechnol. 9, 2709–2716.

Gho, Y., Shin, S.-S., Choi, Y.H., Ko, K., Kim, W.-J., Moon, S.-K., 2019. Hydrangenol suppresses VEGF-stimulated angiogenesis by targeting p27KIP1-dependent G1-cell cycle arrest, VEGFR-2-mediated signaling, and MMP-2 expression. Anim. Cells Syst. 23, 72–81.

Gilman, E.F., 1999. Hydrangea macrophylla. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences - Fact Sheet FPS-258, 1–3.

Giovenzana, V., Beghi, R., Buratti, S., Civelli, R., Guidetti, R., 2014. Monitoring of fresh-cut Valerianella locusta Laterr. shelf life by electronic nose and VIS-NIR spectroscopy. Talanta 120, 368–375.

Gitelson, A.A., Merzlyak, M.N., Chivkunova, O.B., 2001. Optical Properties and Nondestructive Estimation of Anthocyanin Content in Plant Leaves. Photochem. Photobiol. 74, 38–45.

Gitelson, A.A., Zur, Y., Chivkunova, O.B., Merzlyak, M.N., 2002. Assessing Carotenoid Content in Plant Leaves with Reflectance Spectroscopy. Photochem. Photobiol. 75, 272–281.

Gobbo-Neto, L., Lopes, N.P., 2007. Gobbo-Neto, Leonardo, and Norberto P. Lopes. Medicinal plants: factors of influence on the content of secondary metabolites. Quím. Nova 30, 374–381.

Gong, P., Pu, R., Heald, R.C., 2002. Analysis of in situ hyperspectral data for nutrient estimation of giant sequoia. Int. J. Remote Sens. 23, 1827–1850.

Gotoh, T., Gomi, K., 2000. Population Dynamics of Tetranychus Kanzawai (Acari: Tetranychidae) on Hydrangea. Exp. Appl. Acarol. 24, 337–350.

Goyal, S.K., Samsher, Goyal, R.K., 2010. Stevia (Stevia rebaudiana) a bio-sweetener: a review. Int. J. Food Sci. Nutr. 61, 1–10.

Greer, D.H., 2005. Non-destructive chlorophyll fluorescence and colour measurements of ‘Braeburn’ and ‘Royal Gala’ apple (Malus domestica) fruit development throughout the growing season. N. Z. J. Crop Hortic. 33, 413–421.

Großkinsky, D.K., Pieruschka, R., Svensgaard, J., Rascher, U., Christensen, S., Schurr, U., Roitsch, T., 2015a. Phenotyping in the fields: dissecting the genetics of quantitative traits and digital farming. New Phytol. 207, 950–952.

Großkinsky, D.K., Svensgaard, J., Christensen, S., Roitsch, T., 2015b. Plant phenomics and the need for physiological phenotyping across scales to narrow the genotype-to-phenotype knowledge gap. J. Exp. Bot. 66, 5429–5440.

Haaland, D.M., Thomas, E.V., 1988. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal. Chem. 60, 1193–1202.

Haboudane, D., Miller, J.R., Pattey, E., Zarco-Tejada, P.J., Strachan, I.B., 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. Remote Sens. Environ. 90, 337–352.

Hagan, A.K., Mullen, J.M., 2001. Diseases of Hydrangea. Alabama Cooperative Extension System ANR-1212.

Halcomb, M., Reed, S., 2012. Hydrangea Production. UT Extension, 1–13.

Harborne, J.B., 1973. Phenolic Compounds. In: Harborne, J.B. (Ed.) Phytochemical Methods. Springer, Dordrecht, pp. 33–88.

Hariri, M.R., Balqis, N., 2013. Study of Anthocyanin Pigment in Three Different Sepal Colour of Hortensia (Hydrangea macrophylla). Proceeding ICGRC - 4th International Conference on Global Resource Conservation & 10th Indonesian Society for Plant Taxonomy Congress, 269–273.

Hatfield, J.L., Prueger, J.H., 2010. Value of Using Different Vegetative Indices to Quantify Agricultural Crop Characteristics at Different Growth Stages under Varying Management Practices. Remote Sens. 2, 562–578.

Haworth, M., Marino, G., Centritto, M., 2018. An introductory guide to gas exchange analysis of photosynthesis and its application to plant phenotyping and precision irrigation to enhance water use efficiency. J. Water Clim. Chang. 9, 786–808.

Hester, K.A., Bi, G., Czarnota, M.A., Fulcher, A., Keever, G.J., Lieth, J.H., Orsi, J.D., Whipker, B.E., Sullivan, K., Palmer, C.L., 2013. Impact of Augeo, Configure and Florel on Hydrangea Branching. J. Environ. Hort. 31, 27–29.

Hill, R.A., 1986. Naturally occuring isocoumarins. In: Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products. Springer, Vienna.

Ho, C.-T., 1992. Phenoloc Compounds in Food: An Overview. In: Huang, M.-T., Ho, C.-T., Lee, C.Y. (Eds.) Phenolic Compounds in Food and Their Effects on Health II. American Chemical Society, Washington, DC.

Horler, D., Dockray, M., Barber, J., 1983. The red edge of plant leaf reflectance. Int. J. Remote Sens. 4, 273–288.

Hotelling, H., 1931. The Generalization of Student's Ratio. Ann. Math. Statist. 2, 360–378.

Hu, Y., Zhang, H., Liang, W., Xu, P., Lou, K., Pu, J., 2020. Rapid and Simultaneous Measurement of Praeruptorin A, Praeruptorin B, Praeruptorin E, and Moisture Contents in Peucedani Radix Using Near-Infrared Spectroscopy and Chemometrics. J. AOAC Int. 103, 504–512.

Huang, Y., Dong, W., Sanaeifar, A., Wang, X., Luo, W., Zhan, B., Liu, X., Li, R., Zhang, H., Li, X., 2020. Development of simple identification models for four main catechins and caffeine in fresh green tea leaf based on visible and near-infrared spectroscopy. Comput. Electron. Agric. 173, 105388.

Huda, M.S., Roh, M.S., 2014. Growth and flowering response of woody and herbaceous floral crops grown in petroleum-based plastic pots and bioplastic pots containing poultry feather fibers. Sci. Hortic. 170, 131–136.

Huete, A.R., 2012. Vegetation Indices, Remote Sensing and Forest Monitoring. Geogr. Compass 6, 513–532.

Ibrahim, R.K., Towers, G., 1960. Studies of Hydrangenol in Hydrangea macrophylla Ser.: I. Isolation, Identification, and Biosynthesis From C14-labelled Compounds. Can. J. Biochem. Physiol. 38, 627–634.

Jackson, R.D., Huete, A.R., 1991. Interpreting vegetation indices. Prev. Vet. Med. 11, 185–200.

Jansen, M., Gilmer, F., Biskup, B., Nagel, K.A., Rascher, U., Fischbach, A., Briem, S., Dreissen, G., Tittmann, S., Braun, S., Jaeger, I. de, Metzlaff, M., Schurr, U., Scharr, H., Walter, A., 2009. Simultaneous phenotyping of leaf growth and chlorophyll fluorescence via GROWSCREEN FLUORO allows detection of stress tolerance in Arabidopsis thaliana and other rosette plants. Funct. Plant Biol. 36, 902–914.

Ji, L., Zhang, L., Wylie, B., 2009. Analysis of Dynamic Thresholds for the Normalized Difference Water Index. Photogramm. Eng. Remote Sens. 75, 1307–1317.

Jung, C.-H., Kim, Y., Kim, M.-S., Lee, S., Yoo, S.-H., 2016. The establishment of efficient bioconversion, extraction, and isolation processes for the production of phyllodulcin, a

potential high intensity sweetener, from sweet hydrangea leaves (Hydrangea macrophylla Thunbergii). Phytochemical analysis: PCA 27, 140–147.
Junker, L.V., Ensminger, I., 2016. Fast detection of leaf pigments and isoprenoids for eco-physiological studies, plant phenotyping and validating remote-sensing of vegetation. Physiol. Plant. 158, 369–381.
Kakani, V.G., Reddy, K.R., Zhao, D., Gao, W., 2004. Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. Physiol. Plant. 121, 250–257.
Kakegawa, H., Matsumoto, H., Satoh, T., 1988. Inhibitory Effects of Hydrangenol Derivatives on the Activation of Hyaluronidase and ther Antiallergic Ectivities. Planta Med., 385–389.
Kala, C.P., 2015. Medicinal and aromatic plants: Boon for enterprise development. J. Appl. Res. Med. Aromat. Plants 2, 134–139.
Kamarulzaman, N.H., Jamal, K., Vijayan, G., Ab. Jalil, S.M., 2014. Will Consumers Purchase Stevia as a Sugar Substitute?: An Exploratory Study on Consumer Acceptance. J. Food Prod. Mark. 20, 122–139.
Kamei, K., Matsuoka, H., Furuhata, S.I., Fujisaki, R.I., Kawakami, T., Mogi, S., Yoshihara, H., Aoki, N., Ishii, A., Shibuya, T., 2000. Anti-Malarial Activity of Leaf-Extract of Hydrangea macrophylla, a Common Japanese Plant. Acta Med. Okayama 54, 227–232.
Kawamura, M., Kagata, M., Masaki, E., Nishi, H., 2002. Phyllodulcin, a Constituent of "Amacha", Inhibits Phosphodiesterase in Bovine Adrenocortical Cells. Pharmacol. Toxicol. 90, 106–108.
Kefauver, S.C., Vicente, R., Vergara-Díaz, O., Fernandez-Gallego, J.A., Kerfal, S., Lopez, A., Melichar, J.P.E., Serret Molins, M.D., Araus, J.L., 2017. Comparative UAV and Field Phenotyping to Assess Yield and Nitrogen Use Efficiency in Hybrid and Conventional Barley. Front. Plant Sci. 8, 1733.
Kesumawati, E., Hosokawa, M., Kimata, T., Uemachi, T., Yazawa, S., 2009. Flower greening in phytoplasma-infected Hydrangea macrophylla grown under different shading conditions. Sci. Hortic. 121, 199–205.
Khait, I., Lewin-Epstein, O., Sharon, R., Saban, K., Perelman, R., Boonman, A., Yovel, Y., Hadany, L., 2018. Plants emit informative airborne sounds under stress. bioRxiv 507590.
Khoddami, A., Wilkes, M.A., Roberts, T.H., 2013. Techniques for analysis of plant phenolic compounds. Molecules 18, 2328–2375.
Kim, E., Lim, S.M., Kim, M.S., Yoo, S.H., Kim, Y., 2017. Phyllodulcin, a Natural Sweetener, Regulates Obesity-Related Metabolic Changes and Fat Browning-Related Genes of Subcutaneous White Adipose Tissue in High-Fat Diet-Induced Obese Mice. Nutrients 9, 1–15.
Kim, E., Shin, J.H., Seok, P.R., Kim, M.S., Yoo, S.H., Kim, Y., 2018. Phyllodulcin, a natural functional sweetener, improves diabetic metabolic changes by regulating hepatic lipogenesis, inflammation, oxidative stress, fibrosis, and gluconeogenesis in db/db mice. J. Funct. Foods 42, 1–11.
Kim, H.-J., Kang, C.-H., Jayasooriya, R., Dilshara, M.G., Lee, S., Choi, Y.H., Seo, Y.T., Kim, G.-Y., 2016a. Hydrangenol inhibits lipopolysaccharide-induced nitric oxide production in BV2 microglial cells by suppressing the NF-κB pathway and activating the Nrf2-mediated HO-1 pathway. Int. Immunopharmacol. 35, 61–69.
Kim, M.-J., Yoo, S.-H., Kim, Y., Hong, J.-H., 2016b. Relative sweetness and sweetness quality of phyllodulcin (3R)-8-Hydroxy-3-(3-hydroxy-4-methoxyphenyl)-3,4-dihydro-1H-isochromen-1-one. Food Sci. Biotechnol. 25, 1065–1072.

Kindl, H., 1971. Formation of a Stilbene in Isolated Chloroplasts. Hoppe-Seyler's Z. Physiol. Chem. 325, 767–768.

Koirala, A., Walsh, K.B., Wang, Z., McCarthy, C., 2019. Deep learning – method overview and review of use for fruit detection and yield estimation. Comput. Electron. Agric. 162, 219–234.

Kora, A.J., 2019. Leaves as dining plates, food wraps and food packing material: Importance of renewable resources in Indian culture. Bull. Natl. Res. Cent. 43.

Kumar, A., Saini, G., Nair, A., Sharma, R., 2012. UPLC: A Preeminent Technique In Pharmaceutical Analysis. Acta Pol. Pharm. - Drug Research 69, 371–380.

Landgrebe, D., 1997. On Information Extraction Principles for Hyperspectral Data: A White Paper. School of Electrical and Computer Engineering. Purdue University, West Lafayette.

Lattanzio, V., 2013. Phenolic Compounds: Introduction. In: Ramawat, K.G., Mérillon, J.-M. (Eds.) Natural Products. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1543–1580.

Li, L., Zhang, Q., Huang, D., 2014. A review of imaging techniques for plant phenotyping. Sensors 14, 20078–20111.

Li, Y., Mmbaga, M.T., Zhou, B., Joshua, J., Rotich, E., Parikh, L., 2016. Diseases of Hydrangea. In: McGovern, R.J., Elmer, W.H. (Eds.) Handbook of Florists' Crops Diseases. Springer International Publishing, Cham, pp. 1–19.

Li, Z., Guo, R., Li, M., Chen, Y., Li, G., 2020. A review of computer vision technologies for plant phenotyping. Comput. Electron. Agric. 176, 105672.

Loconsole, D., Santamaria, P., 2021. UV Lighting in Horticulture: A Sustainable Tool for Improving Production Quality and Food Safety. Horticulturae 7, 1–13.

Lu, S., 2013. Effects of Leaf Surface Wax on Leaf Spectrum and Hyperspectral Vegetation Indices. IGARSS 2013 - 2013 IEEE International Geoscience and Remote Sensing Symposium.

Lu, X., Lu, S., 2015. Effects of adaxial and abaxial surface on the estimation of leaf chlorophyll content using hyperspectral vegetation indices. Int. J. Remote Sens. 36, 1447–1469.

Lubbe, A., Verpoorte, R., 2011. Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind. Crops Prod. 34, 785–801.

Luhmer, K., Schulze-Kaysers, N., Feuereisen, M., Wirth, L., Maretzky, F., Wüst, M., Blum, H., Dörr, E., Pude, R., 2021. Fatty Acid Composition, Tocopherols, Volatile Compounds, and Sensory Evaluation of Low Morphine Yielding Varieties of Poppy (Papaver somniferum L.) Seeds and Oils. J. Agric. Food Chem. 69, 3439–3451.

Luo, J., Ying, K., He, P., Bai, J., 2005. Properties of Savitzky–Golay digital differentiators. Digit. Signal Process. 15, 122–136.

Ma, K.C., Choi, D.S., Ko, S.J., Kim, H.J., Kim, D.I., Kim, H.W., Kim, S.S., 2015. The Seasonal Occurrence and Selecting a Pesticide Against Major Insect Pests Occurring in Greenhouse-grown Hydrangea in Jeonnam Province. J. Agric. Sci. Technol. 50, 33–38.

Mach, B.M., Potter, D.A., 2018. Quantifying bee assemblages and attractiveness of flowering woody landscape plants for urban pollinator conservation. PloS one 13, e0208428.

Mahdavian, K., Ghorbanli, M., Kalantari, K., 2008. The Effects of Ultraviolet Radiation on the Contents of Chlorophyll, Flavonoid, Anthocyanin and Proline in Capsicum annuum L. Turk. J. Bot. 32, 25–33.

Mahlein, A.-K., 2016. Plant Disease Detection by Imaging Sensors - Parallels and Specific Demands for Precision Agriculture and Plant Phenotyping. Plant Dis. 100, 241–251.

Mahlein, A.-K., Kuska, M.T., Behmann, J., Polder, G., Walter, A., 2018. Hyperspectral Sensors and Imaging Technologies in Phytopathology: State of the Art. Annu. Rev. Phytopathol. 56, 535–558.

Mahlein, A.-K., Kuska, M.T., Thomas, S., Wahabzada, M., Behmann, J., Rascher, U., Kersting, K., 2019. Quantitative and qualitative phenotyping of disease resistance of crops by hyperspectral sensors: seamless interlocking of phytopathology, sensors, and machine learning is needed! Curr. Opon. Plant Biol. 50, 156–162.

Mani, J.S., Johnson, J.B., Steel, J.C., Broszczak, D.A., Neilsen, P.M., Walsh, K.B., Naiker, M., 2020. Natural product-derived phytochemicals as potential agents against coronaviruses: A review. Virus Res. 284, 197989.

Mardones, M., Trampe-Jaschik, T., Hofmann, T.A., Mangelsdorff, R.D., Piepenbring, M., 2018. New neotropical species of Phyllachorales based on molecular, morphological, and ecological data. Mycologia 110, 835–859.

Marwood, C.A., Greenberg, B.M., 1996. Effect of Supplementary UVB Radiation on Chlorophyll Synthesis and Accumulation of Photosystems during Chloroplast Development in Spirodela oligorrhiza. Photochem. Photobiol. 64, 664–670.

Masuka, B., Araus, J.L., Das, B., Sonder, K., Cairns, J.E., 2012. Phenotyping for abiotic stress tolerance in maize. J. Integr. Plant Biol. 54, 238–249.

Matsuno, T., Kunikate, T., Tanigawa, T., Suyama, T., Yamada, A., 2008. Establishment of Cultivation System Based on Flowering Characteristics in Hydrangea serrata (Thunb.) Ser. Hort. Res. (Japan) 7, 189–195.

McClintock, 1957. A Monograph of the Genus Hydrangea. Proceedings of the California Academy of Sciences, 4th series 29, 147–256.

McGuire, R.G., 1992. Reporting of Objective Color Measurements. HortScience 27, 1254–1255.

Meeboon, J., Takamatsu, S., 2015. Notes on powdery mildews (Erysiphales) in Japan: II. Erysiphe sect. Microsphaera. Mycoscience 56, 230–236.

Merzlyak, M.N., Gitelson, A.A., Chivkumova, O.B., Rakitin, V.Y., 1999. Non-destructive optical detection of pigment changes during leaf senescence and fruit ripening. Physiol. Plant. 106, 135–141.

Midcap, J.T., 2005. Accelerated Production of Forced Remontant Hydrangeas Under Nursery Conditions. SNA Research Conference 50, 84–86.

Mishra, K.B., Mishra, A., Klem, K., Govindjee, 2016. Plant phenotyping: a perspective. Ind. J. Plant Physiol. 21, 514–527.

Mittal, R., Singh, S.P., 2007. Shifting from Agriculture to Agribusiness: The Case of Aromatic Plants. Agric. Econ. Res. Rev. 20, 541–550.

Moll, L., Wever, C., Völkering, G., Pude, R., 2020. Increase of Miscanthus Cultivation with New Roles in Materials Production—A Review. Agronomy 10, 308.

Moll, M.D., Kahlert, L., Blings, M., Schwarze, E.-C., Hillebrand, S., Ley, J., Kraska, T., Pude, R., 2021a. Einfluss verschiedener Nacherntеverfahren auf wertgebende Inhaltsstoffe von Teehortesien (Hydrangea macrophylla subsp. serrata). In: Stützel, H., Fricke, A. (Eds.) Mitteilungen der Gesellschaft für Pflanzenwissenschaften, Band 32. CLOSING THE CYCLE - Pflanze und Tier im Agrarsystem, pp. 233–234.

Moll, M.D., Vieregge, A.S., Wiesbaum, C., Blings, M., Vana, F., Hillebrand, S., Ley, J., Kraska, T., Pude, R., 2021b. Dihydroisocoumarin Content and Phenotyping of Hydrangea macrophylla subsp. serrata Cultivars under Different Shading Regimes. Agronomy 11.

Morel, P., 2001. Growth Control Of Hydrangea Macrophylla Through Water Restriction. Acta Hortic., 51–58.

Morisse, M., Wells, D.M., Millet, E.J., Lillemo, M., Fahrner, S., Cellini, F., Lootens, P., Muller, O., Herrera, J.M., Bentley, A.R., Janni, M., 2022. A European perspective on opportunities and demands for field-based crop phenotyping. Field Crops Res. 276, 108371.
Müller-Xing, R., Xing, Q., Goodrich, J., 2014. Footprints of the sun: memory of UV and light stress in plants. Front. Plant Sci. 5, 474.
Muraya, M.M., Chu, J., Zhao, Y., Junker, A., Klukas, C., Reif, J.C., Altmann, T., 2017. Genetic variation of growth dynamics in maize (Zea mays L.) revealed through automated non-invasive phenotyping. Plant J. 89, 366–380.
Myung, D.-B., Han, H.-S., Shin, J.-S., Park, J.Y., Hwang, H.J., Kim, H.J., Ahn, H.S., Lee, S.H., Lee, K.-T., 2019. Hydrangenol Isolated from the Leaves of Hydrangea serrata Attenuates Wrinkle Formation and Repairs Skin Moisture in UVB-Irradiated Hairless Mice. Nutrients 11.
Nakajima, S., Sugiyama, S., Suto, M., 1979. Synthesis of Antifungal Isocoumarins (I). Organic Preparations and Procedures International 11, 77–86.
Nakashima, C., Oetari, A., Kanti, A., Saraswati, R., Widyastuti, Y., Ando, K., 2010. New species and newly recorded species of Cercospora and allied genera from Indonesia. Mycosphere 1, 315–323.
Neacsu, N.A., Madar, A., 2014. Artificial sweeteners versus natural sweeteners. Bull. Transilv. Univ. Bras. V: Econ. Sci. 7, 59–64.
Negbi, M., 2005. Saffron cultivation: past, present and future prospects. In: Negbi, M. (Ed.) Saffron - Crocus sativus L. Taylor & Francis e-Library.
Nesi, B., Lazzareschi, S., Pecchioli, S., Grassotti, A., Salazar-Orozco, G., 2013. Effects of Colored Shade Netting on the Vegetative Development and on the Photosynthetic Activity in Several Hydrangea Genotypes. Acta Hort. 1000.
Niederbacher, B., Winkler, J.B., Schnitzler, J.P., 2015. Volatile organic compounds as non-invasive markers for plant phenotyping. J. Exp. Bot. 66, 5403–5416.
Nissim-Levi, A., Farkash, L., Hamburger, D., Ovadia, R., Forrer, I., Kagan, S., Oren-Shamir, M., 2008. Light-scattering shade net increases branching and flowering in ornamental pot plants. J. Hortic. Sci. Biotechnol. 83, 9–14.
Noland, R.L., Wells, M.S., Coulter, J.A., Tiede, T., Baker, J.M., Martinson, K.L., Sheaffer, C.C., 2018. Estimating alfalfa yield and nutritive value using remote sensing and air temperature. Field Crops Res. 222, 189–196.
Noodén, L.D., Guiamét, J.J., John, I., 2004. 15. Whole Plant Senescence, 228–244.
Nordli, E.F., Strøm, M., Torre, S., 2011. Temperature and photoperiod control of morphology and flowering time in two greenhouse grown Hydrangea macrophylla cultivars. Sci. Hortic. 127, 372–377.
Nováková, L., Matysová, L., Solich, P., 2006. Advantages of application of UPLC in pharmaceutical analysis. Talanta 68, 908–918.
Nozawa, K., Yamada, M., Tsuda, Y., Kawai, K.-I., Nakajima, S., 1981. Antifungal Activity of Oosponol, Oospolactone, Phyllodulcin, Hydrangenol, and Some Other Related Compounds. Chem. Pharm. Bull. 29, 2689–2691.
O'Meara, L., Chappell, M.R., van Iersel, M.W., 2014. Water Use of Hydrangea macrophylla and Gardenia jasminoides in Response to a Gradually Drying Substrate. HortScience 49, 493–498.
Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43.
O'Haver, T.C., 1979. Potential Clinical Applications of Derivative and Wavelength-Modulation Spectrometry. Clin. Chem. 25, 1548–1553.

Ohba, H., Akiyama, S., 2013. A Revision of the Species of Hydrangea (Hydrangeaceae) described by Siebold and Zuccarini, Part 1. Bull. Natl. Mus. Nat. Sci., Ser. B 39, 173–194.

Ohba, H., Akiyama, S., 2014. A Revision of the Species of Hydrangea (Hydrangeaceae) described by Siebold and Zuccarini, Part 2. Bull. Natl. Mus. Nat. Sci., Ser. B 40, 29–45.

O'Meara, L., van Iersel, M.W., Chappell, M.R., 2013. Modeling Daily Water Use of Hydrangea macrophylla and Gardenia jasminoides as Affected by Environmental Conditions. HortScience 48, 1040–1046.

Owen, J.S., Fulcher, A., LeBude, A., Chappell, M., 2016. Hydrangea Production: Cultivar Selection and General Practices to Consider When Propagating and Growing Hydrangea. UT Extension PB 1840-A, 1–12.

Owusu, P.A., Asumadu-Sarkodie, S., 2016. A review of renewable energy sources, sustainability issues and climate change mitigation. Cogent Engin. 3, 1167990.

Özcan, M., 2002. Nutrient composition of rose (Rosa canina L.) seed and oils. J. Med. Food 5, 137–140.

Pagter, M., Liu, F., Jensen, C.R., Petersen, K.K., 2008. Effects of chilling temperatures and short photoperiod on PSII function, sugar concentrations and xylem sap ABA concentrations in two Hydrangea species. Plant Sci. 175, 547–555.

Pal, S., Chatare, V., Pal, M., 2011. Isocoumarin and Its Derivatives: An Overview on their Synthesis and Applications. Curr. Org. Chem. 15, 782–800.

Pantazi, X.E., Moshou, D., Alexandridis, T., Whetton, R.L., Mouazen, A.M., 2016. Wheat yield prediction using machine learning and advanced sensing techniques. Comput. Electron. Agric. 121, 57–65.

Park, J.H., Shin, H.D., 2016. *Ramularia hydrangeicola* sp. nov. with distinctive traits on *Hydrangea serrata* f. *acuminata* in Korea. Mycotaxon 131, 95–102.

Park, Y.-J., Kim, H.-K., 1993. Studies on Factors Indfluencing the Rooting of Hydrangea serrata Seringe var. Oamacha Honda Cutting. J. East Coast. Res. 4, 61–69.

Parker, M.N., Lopetcharat, K., Drake, M.A., 2018. Consumer acceptance of natural sweeteners in protein beverages. J. Dairy Sci. 101, 8875–8889.

Peñuelas, J., Baret, F., Filella, I., 1995. Semi-empirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. Photosynthetica 31, 221–230.

Peñuelas, J., Gamon, J.A., Fredeen, A.L., Merino, J., Field, C.B., 1994. Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. Remote Sensing of Environment 48, 135–146.

Pieruschka, R., Schurr, U., 2019. Plant Phenotyping: Past, Present, and Future. Plant phenomics 2019, 7507131.

Pissard, A., Baeten, V., Dardenne, P., Dupont, P., Mateur, M., 2018. Use of NIR specroscopy on fresh apples to determine the phenolic compounds and dry matter in peel and flesh. Biotechnol. Agron. Soc. Environ. 22, 3–12.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional plant biology: FPB 39, 839–850.

Qiu, R., Wei, S., Zhang, M., Li, H., Sun, H., Liu, G., Li, M., 2018. Sensors for measuring plant phenotyping: A review. Int. J. Agric. Biol. 11, 1–17.

Ramakrishna, A., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6, 1720–1731.

Rao, M.R., Palada, M.C., Becker, B.N., 2004. Medicinal and aromatic plants in agroforestry systems. Agrofor. Syst. 61-62, 107–122.

Rascher, U., Blossfeld, S., Fiorani, F., Jahnke, S., Jansen, M., Kuhn, A.J., Matsubara, S., M Rtin, L.L.A., Merchant, A., Metzner, R., M Ller-Linow, M., Nagel, K.A., Pieruschka, R., Pinto, F., Schreiber, C.M., Temperton, V.M., Thorpe, M.R., van Dusschoten, D., van Volkenburgh, E., Windt, C.W., Schurr, U., 2011. Non-invasive approaches for phenotyping of enhanced performance traits in bean. Funct. Plant Biol. 38, 968–983.

Reed, S.M., 2000. Compatibility Studies in Hydrangea. J. Environ. Hort. 18, 29–33.

Reed, S.M., 2005. Pollination Biology of Hydrangea macrophylla. HortScience 40, 335–338.

Reed, S.M., Rinehart, T.A., 2006. Hydrangea macrophylla and serrata - Should we Lump'em or Split'em. SNA Research Conference 51.

Riahi, S., Pourbasheer, E., Ganjali, M.R., Norouzi, P., 2009. Investigation of different linear and nonlinear chemometric methods for modeling of retention index of essential oil components: concerns to support vector machine. J. Hazard. Mater. 166, 853–859.

Ribaudo, M.O., Hoag, D.L., Smith, M.E., Heimlich, R., 2001. Environmental indices and the politics of the Conservation Reserve Program. Ecol. Indic. 1, 11–20.

Rinaldi, S., Lucia, B. de, Salvati, L., Rea, E., 2014. Understanding complexity in the response of ornamental rosemary to different substrates: A multivariate analysis. Sci. Hortic. 176, 218–224.

Rinehart, T.A., Reed, S.M., Owings, A., Bi, G., 2008. Hydrangea macrophylla Sun Tolerance for the Deep South. SNA Research Conference 53, 198–200.

Roberts, D.A., Roth, K.L., Perroy, R.L., 2011. Hyperspectral Vegetation Indices. In: Thenkabail, A., Lyon, P., Huete, J. (Eds.) Hyperspectral Remote Sensing of Vegetation, vol. 1. CRC Press, pp. 309–328.

Rosenqvist, E., Großkinsky, D.K., Ottosen, C.-O., van de Zedde, R., 2019. The Phenotyping Dilemma-The Challenges of a Diversified Phenotyping Community. Front. Plant Sci. 10, 163.

Rouphael, Y., Kyriacou, M.C., Petropoulos, S.A., Pascale, S. de, Colla, G., 2018. Improving vegetable quality in controlled environments. Sci. Hortic. 234, 275–289.

Rouse, J.W., Haas, R., Schell, J.A., Deering, D.W., Harlan, J.C., 1974. Monitoring the vernal advancement and retrogradation (Greenwave effect) of natural vegetation. NASA/GSFCT Type III final Report, Greenbelt, MD, USA.

Ruett, M., Junker-Frohn, L.V., Siegmann, B., Ellenberger, J., Jaenicke, H., Whitney, C., Luedeling, E., Tiede-Arlt, P., Rascher, U., 2022. Hyperspectral imaging for high-throughput vitality monitoring in ornamental plant production. Sci. Hortic. 291.

Ryu, M.-J., Park, B.-M., Bae, J.H., 2010. Effects of IBA, and NAA on the Rooting of Wild Hydrangea serrata for. acuminata. J. Bio-Env. Con. 19, 397–402.

Saddiqa, A., Usman, M., Cakmak, O., 2017. Isocoumarins and 3,4-dihydroisocoumarins, amazing natural products: a review. Turk. J. Chem. 41, 153–178.

Saeed, A., 2016. Isocoumarins, miraculous natural products blessed with diverse pharmacological activities. Eur. J. Med. Chem. 116, 290–317.

Saharudin, A.M.B., Nazri, N.B.M., Hawi, M.H.B., Mar, S.O., 2020. Acceptance of Stevia as a Sugar Substitute and its Determinants among Health Educated Individuals and its Determinants. Curr. Res. Nutr. Food Sci. 8, 226–237.

Sanaeifar, A., Huang, X., Chen, M., Zhao, Z., Ji, Y., Li, X., He, Y., Zhu, Y., Chen, X., Yu, X., 2020. Nondestructive monitoring of polyphenols and caffeine during green tea processing using Vis-NIR spectroscopy. Food Sci. Nutr. 8, 5860–5874.

Sanjeewani, W.C., Sutharsan, S., Srikrishnah, S., 2019. Effects of Hydrangea (Hydrangea macrophylla L.) Leaf Extract Foliar Application on Growth and Yield of Mungbean (Vigna radiata L.). Int. J. Botany Studies 4, 180–184.

Sarijeva, G., Knapp, M., Lichtenthaler, H.K., 2007. Differences in photosynthetic activity, chlorophyll and carotenoid levels, and in chlorophyll fluorescence parameters in green sun and shade leaves of Ginkgo and Fagus. J. Plant. Physiol. 164, 950–955.

Saviano, A.M., Madruga, R.O.G., Lourenço, F.R., 2015. Measurement uncertainty of a UPLC stability indicating method for determination of linezolid in dosage forms. Measurement 59, 1–8.

Savita, S.M., Sheela, K., Sunanda, S., Shankar, A.G., Ramakrishna, P., 2004. Stevia rebaudiana – A Functional Component for Food Industry. J. Hum. Ecol. 15, 261–264.

Savithramma, N., Linga Rao, M., Suhrulatha, D., 2011. Screening of Medicinal Plants for Secondary Metabolites. Middle-East J. Sci. Res. 8, 579–584.

Savitzky, A., Golay, M., 1964. Smoothing and Differentiation of Data by Simplified Least Squares Procedures. Anal. Chem. 36, 1627–1639.

Scharr, H., Minervini, M., French, A.P., Klukas, C., Kramer, D.M., Liu, X., Luengo, I., Pape, J.-M., Polder, G., Vukadinovic, D., Yin, X., Tsaftaris, S.A., 2015. Leaf segmentation in plant phenotyping: a collation study. Mach. Vis. Appl. 27, 585–606.

Schulz, H., 2004. Analysis of Coffee, Tea, Cocoa, Tobacco, Spices, Medicinal and Aromatic Plants, and Related Products. In: Roberts, C.A., Workman Jr., J., Reeves III, J.B. (Eds.) Near-Infrared Spectroscopy in Agriculture, vol. 44. American Society of Agronomy, Madison, WI, pp. 345–376.

Schulz, H., Drews, H.-H., Krüger, H., 1999. Rapid NIRS Determination of Quality Parameters in Leaves and Isolated Essential Oils of Mentha Species. J. Essent. Oil Res. 11, 185–190.

Shabir, G., Saeed, A., El-Seedi, H.R., 2021. Natural isocoumarins: Structural styles and biological activities, the revelations carry on … Phytochemistry 181.

Shakoor, N., Lee, S., Mockler, T.C., 2017. High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field. Curr. Opon. Plant Biol. 38, 184–192.

Shin, J.-S., Han, H.-S., Lee, S.-B., Myung, D.-B., Lee, K., Lee, S.H., Kim, H.J., Lee, K.-T., 2019. Chemical Constituents from Leaves of Hydrangea serrata and Their Anti-photoaging Effects on UVB-Irradiated Human Fibroblasts. Biol. Pharm. Bull. 42, 424–431.

Shin, S.-S., Ko, M.-C., Park, Y.-J., Hwang, B., Park, S.L., Kim, W.-J., Moon, S.-K., 2018. Hydrangenol inhibits the proliferation, migration, and invasion of EJ bladder cancer cells via p21WAF1-mediated G1-phase cell cycle arrest, p38 MAPK activation, and reduction in Sp-1-induced MMP-9 expression. EXCLI J. 17, 531–543.

Shin, W., Kim, S.J., Shin, J.M., Kim, S.H., 1995. Structure-taste correlations in sweet dihydrochalcone, sweet dihydroisocoumarin, and bitter flavone compounds. J. Med. Chem. 38, 4325–4331.

Siegrist, M., Hartmann, C., 2020. Consumer acceptance of novel food technologies. Nat. Food 1, 343–350.

Simmons, E.L., 1975. Diffuse reflectance spectroscopy: a comparison of the theories. Appl. Opt. 14, 1380–1386.

Singh, A.K., Ganapathysubramanian, B., Sarkar, S., Singh, A., 2018. Deep Learning for Plant Stress Phenotyping: Trends and Future Perspectives. Trends Plant Sci. 23, 883–898.

Soheila, A.-H.-M., 2000. Plant responses to ultraviolet-B (UV-B: 280-320 nm) stress: What are the key regulators? Plant Growth Regul. 32, 27–39.

Souza, R. de, Buchhart, C., Heil, K., Plass, J., Padilla, F.M., Schmidhalter, U., 2021. Effect of Time of Day and Sky Conditions on Different Vegetation Indices Calculated from Active and Passive Sensors and Images Taken from UAV. Remote Sens. 13, 1691.

Steyn, W.J., Wand, S., Holcroft, D.M., Jacobs, G., 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155, 349–361.
Stigter, C.J., 1984. Shading: a traditional method of microclimate manipulation. Neth. J. Agri. Sci. 32, 81–86.
Strid, A., Porra, R.J., 1992. Alterations in Pigment Content in Leaves of Pisum sativum After Exposure to Supplementary UV-B. Plant Cell Physiol. 33, 1015–1023.
Stroppiana, D., Migliazzi, M., Chiarabini, V., Crema, A., Musanti, M., Franchino, C., Villa, P., 2015. Rice Yield Estimation Using Multispectral Data From UAV: A Preliminary Experiment In Northern Italy. IGARSS, 4664–4667.
Struckmeyer, B.E., 1950. Blossom Bud Induction and Differentiation in Hydrangea. J. Am. Soc. Hortic. Sci., 410–414.
Stuber, C.W., Polacco, M., Lynn, M., 1999. Synergy of Empirical Breeding, Marker-Assisted Selection, and Genomics to Increase Crop Yield Potential. Crop Sci. 39, 1571–1583.
Sun, Y., Bi, G., Niu, G., Perez, C., 2015. Foliar Application of Dikegulac Sodium Increases Branching of ‘Merritt’s Supreme’ Bigleaf Hydrangea. hortte 25, 306–312.
Surjadinata, B.B., Jacobo-Velázquez, D.A., Cisneros-Zevallos, L., 2017. UVA, UVB and UVC Light Enhances the Biosynthesis of Phenolic Antioxidants in Fresh-Cut Carrot through a Synergistic Effect with Wounding. Molecules 22.
Suyama, T., Tanigawa, T., Yamada, A., Matsuno, T., Kunitake, T., Saeki, K., Nakamura, C., 2015. Inheritance of the Double-flowered Trait in Decorative Hydrangea Flowers. Hortic. J. 84, 253–260.
Suzuki, H., Ikeda, T., Matsumoto, T., Noguchi, M., 1977. Isolation and Identification of a New Glycoside, Phyllodulcin-8-O-β- d -glucose from the Cultured Cells and Fresh Leaves of Amacha (Hydrangea macrophylla Seringe var. Thunbergii Makino). Agric. Biol. Chem. 41, 1815–1817.
Suzuki, H., Ikeda, T., Matsumoto, T., Noguchi, M., 1978. Polyphenol Components in Cultured Cells of Amacha (Hydrangea macrophylla Seringe var. Thunbergii Makino). Agric. Biol. Chem. 42, 1133–1137.
Swartz, M.E., 2005. UPLC ™ : An Introduction and Review. J. Liq. Chromatogr. Relat. Technol. 28, 1253–1263.
Tachibana, Y., Hashimoto, Y., Hagiwara, Y., Konishii, T., Kurokawa, N., 1974. The Quantitative Analysis of Phyllodulcin in "Amacha" (Sweet Hydrangea) by Means of Thin-LAyer Chromatography. Yakugaku Zasshi 94, 1167–1169.
Takhtajan, A., 2009. Flowering Plants. Springer Netherlands, Dordrecht, 40 pp.
Tanaka, H., 1982. Steviol-glycosides: new natural sweeteners. Trends Analyt. Chem. 1, 246–248.
Tavoni, A., Schlüter, M., Levin, S., 2012. The survival of the conformist: social pressure and renewable resource management. J. Theor. Biol. 299, 152–161.
Thenot, F., Méthy, M., Winkel, T., 2002. The Photochemical Reflectance Index (PRI) as a water-stress index. Int. J. Remote Sens. 23, 5135–5139.
Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., Deerberg, G., 2020. Effects of Light on Secondary Metabolites in Selected Leafy Greens: A Review. Front. Plant Sci. 11, 497.
Thomas, S., Behmann, J., Steier, A., Kraska, T., Muller, O., Rascher, U., Mahlein, A.-K., 2018. Quantitative assessment of disease severity and rating of barley cultivars based on hyperspectral imaging in a non-invasive, automated phenotyping platform. Plant methods 14, 1–12.

Tsaftaris, S.A., Noutsos, C., 2009. Plant Phenotyping with Low Cost Digital Cameras and Image Analytics. In: Athanasiadis, I.N., Rizzoli, A.E., Mitkas, P.A., Gómez, J.M. (Eds.) Information Technologies in Environmental Engineering. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 238–251.

Tschannerl, J., Ren, J., Jack, F., Krause, J., Zhao, H., Huang, W., Marshall, S., 2019. Potential of UV and SWIR hyperspectral imaging for determination of levels of phenolic flavour compounds in peated barley malt. Food Chem. 270, 105–112.

Udelhoven, T., Jarmer, T., Hostert, P., Hill, J., 2001. The aquisition of spectral reflectance measurements under field and laboratory conditions as support for hyperspectral applications in precision farming. Proc. 2nd EARSeL Workshop on Imaging Spectroscopy, Enschede, 11.-13- July 2000 (F. van der Meer, A. Skidmore & W. Bakker, Eds.).

Ujihara, M., Shinozaki, M., Kato, M., 1995. Accumulation of Phyllodulcin in Sweet-Leaf Plants of Hydrangea serrata and Its Neutrality in the Defence Against a Specialist Leafmining Herbivore. Res. Popul. Ecol. 37, 249–257.

Ulloa, A.M., 2018. The aesthetic life of artificial flavors. Senses Soc. 13, 60–74.

Vaishnav, P., Demain, A.L., 2011. Unexpected applications of secondary metabolites. Biotechnol. Adv. 29, 223–229.

van Gelderen, C.J., van Gelderen, D.M., 2004. Encyclopedia of Hydrangeas. Timber Press, Portland, Oregon.

Velderrain-Rodríguez, G.R., Palafox-Carlos, H., Wall-Medrano, A., Ayala-Zavala, J.F., Chen, C.-Y.O., Robles-Sánchez, M., Astiazaran-García, H., Alvarez-Parrilla, E., González-Aguilar, G.A., 2014. Phenolic compounds: their journey after intake. Food Funct. 5, 189–197.

Vidalie, H., 1986. Influence of various cold treatments on the reaction to forcing of miniature hydrangea macrophylla. Acta Hortic., 263–267.

Wahabzada, M., Mahlein, A.-K., Bauckhage, C., Steiner, U., Oerke, E.-C., Kersting, K., 2016. Plant Phenotyping using Probabilistic Topic Models: Uncovering the Hyperspectral Language of Plants. Sci. Rep. 6, 22482.

Wallace, M.B., Wax, A., Roberts, D.N., Graf, R.N., 2009. Reflectance spectroscopy. Gastroenterol. Clin. North Am. 19, 233–242.

Walter, A., Liebisch, F., Hund, A., 2015. Plant phenotyping: from bean weighing to image analysis. Plant 11, 14.

Weiler, T.C., 1980. Hydrangeas. In: Larson, R.A. (Ed.) Introduction to Floriculture. Academic Press, London, Oxford, Boston, New York, San Diego, pp. 353–372.

Windham, M.T., Reed, S.M., Mmbaga, M.T., Windham, A.S., Li, Y., Rinehart, T.A., 2011. Evaluation of Powdery Mildew Resistance in Hydrangea macrophylla. J. Environ. Hort. 29, 60–64.

Wold, S., Johansson, E., Cocchi, M., 1993. PLS: partial least squares projections to latent structures. In: Kubinyi, H. (Ed.) 3D QSAR in Drug Design: Theory, Methods and Applications. ESCOM, Leiden, pp. 523–550.

Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemom. Intell. Lab. Syst. 58, 109–130.

Wong Sato, A.A., Kato, M., 2019. Pollination-related functions of decorative sterile flowers of nine Japanese Hydrangea species (Hydrangeaceae). Botany 97, 521–528.

Wulandari, L., Permana, B.D., Kristiningrum, N., 2020. Determination of Total Flavonoid Content in Medicinal Plant Leaves Powder Using Infrared Spectroscopy and Chemometrics. Indones. J. Chem. 20, 1044.

Xue, J., Su, B., 2017. Significant Remote Sensing Vegetation Indices: A Review of Developments and Applications. J. Sensors 2017, 1–17.

Yadav, A.K., Singh, S., Dhyani, D., Ahuja, P.S., 2011. A review on the improvement of stevia [Stevia rebaudiana (Bertoni)]. Can. J. Plant Sci. 91, 1–27.
Yagi, A., Ogata, Y., Yamauchi, T., Nishoka, I., 1977. Metabolism of Phenylpropanoids in Hydrangea serrata var. thunbergii and the Biosynthesis of Phyllodulcin. Phytochemistry 16, 1098–1100.
Yamahara, J., Matsuda, H., Shimoda, H., Ishikawa, H., Kawamori, S., Wariishi, N., Harada, E., Murakami, N., Yoshikawa, M., 1994. Development of bioactive functions in hydrangeae dulcis folium. II. Antiulcer, antiallergy, and cholagoic effects of the extract from hydrangeae dulcis folium. Yakugaku Zasshi 114, 401–413.
Yamashita, H., Sonobe, R., Hirono, Y., Morita, A., Ikka, T., 2020. Dissection of hyperspectral reflectance to estimate nitrogen and chlorophyll contents in tea leaves based on machine learning algorithms. Sci. Rep. 10, 17360.
Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., Wang, Q., 2018. Response of Plant Secondary Metabolites to Environmental Factors. Molecules 23.
Yasuda, T., Kayaba, S., Takahashi, K., Nakazawa, T., Ohsawa, K., 2004. Metabolic fate of orally administered phyllodulcin in rats. J. Nat. Prod. 67, 1604–1607.
Yeary, W.M., Fulcher, A., 2013. A Tennessee Landscape Contractors Guide to Hydrangeas. UT Extension W 304.
Yeh, D.M., Chiang, H.H., 2001. Growth and flower initiation in hydrangea as affected by root restriction and defoliation. Sci. Hortic. 91, 123–132.
Yin, L.Q., Hu, Y.H., Tang, G., Huang, W.C., Li, S.Z., Wang, X.Q., 2010. In vitro culture and rapid propagation of Hydrangea serrata 'Preziosa'. Acta Agric. Shanghai 26, 38–41.
Yoshikawa, M., Yokoyama, T., 1992. Thedgonia ligustrina on Ligustrum japonicum and Cercospora kyotensis sp. nov. on Hydrangea serrata var. thunbergii. Trans. Mycol. Soc. Japan 33, 177–184.
Zarco-Tejada, P.J., Berjón, A., López-Lozano, R., Miller, J.R., Martín, P., Cachorro, V., González, M.R., Frutos, A. de, 2005. Assessing vineyard condition with hyperspectral indices: Leaf and canopy reflectance simulation in a row-structured discontinuous canopy. Remote Sens. Environ. 99, 271–287.
Zhao, H., Chen, X., 2005. Use of Normalized Difference Bareness Index in Quickly Mapping Bare Areas from TM/ETM+. IEEE Operations Center, 3 pp.
Zhou, T.S., Hara, N., 1988. Development of shoot inHydrangea macrophylla I. Terminal and axillary buds. Bot. Mag. Tokyo 101, 281–291.