Invasive Alien Vines Affect Leaf Traits of Riparian Woody Vegetation

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Abstract: The vines Echinocystis lobata and Parthenocissus quinquefolia are spreading over the natural vegetation in riparian zones, which may significantly affect riparian vegetation properties and the quality of litter for aquatic organisms. We examined leaf morphological, biochemical and optical traits of these invasive alien species, each paired with its host, the willows Salix caprea and S. fragilis, respectively. The vines altered the host radiation environment and the amount of photosynthetic pigments. Both vines had significantly higher specific leaf area and lower leaf tissue density compared to the willows, even though the leaves of P. quinquefolia were significantly thicker. Leaf optical properties varied significantly between vines and willows in some spectral regions. Compared to the willows, the vines reflected less light as UV, and more as green, and transmitted more light as green, yellow and red. The overgrowth of the willows with vines affected the reflectance of the willow leaves. Redundancy analysis of the relationships between leaf biochemical traits and reflectance spectra showed that chlorophyll a, anthocyanins, and UVB- and UVA-absorbing substances explained 45% of the reflectance spectra variability, while analysis with morphological traits revealed that specific leaf area, leaf thickness and upper cuticle thickness explained 43%. For leaf transmittance, UVB- and UVA-absorbing substances, carotenoids and anthocyanins explained 53% of the transmittance spectra variability, while analysis with morphological traits revealed that specific leaf area explained 51%. These data show that invasive alien vines can be discerned from each other and their hosts by their spectral signatures. In addition, the differences in the leaf functional traits between the vines and their hosts indicate significant differences in the quality of the plant litter entering the river.

Keywords: invasive alien species; Echinocystis lobata; Parthenocissus quinquefolia; willows; riparian zones; leaf traits

1. Introduction

Riparian zones of woody vegetation have significant roles in the structure and function of aquatic ecosystems, as they represent the most fertile and productive parts of a landscape [1,2]. To protect the multiple ecosystem services of riparian zones, landscape managers need to maintain healthy riparian zones and restore disturbed ones [3]. The vegetation type and width of riparian zones are closely related to the ecological status of the rivers [4–7]. The water regime of rivers strongly affects the riparian vegetation, in terms of the transport of propagules, the frequency and intensity of flooding and water availability [8]. Disturbances to riparian zones increase their vulnerability to invasion by invasive alien plant species [9–11]. These plants mainly colonize riparian zones where the original vegetation has been removed [12]. When established in a catchment area, invasive alien species can exploit natural floods and anthropogenic disturbances to spread further [9].
Invasive alien plants generally have negative impacts upon native plants and ecosystems [2,10]. They affect ecosystems via the flow of energy and the cycling of matter, through changes to the habitat characteristics, excessive use of resources, accumulation of litter and changes to the erosion dynamics [13]. The arrival, establishment, persistence and proliferation of invasive alien plants can reduce the resilience of riparian zones [9]. Their negative effects on the species richness of riparian vegetation are related to moderate disturbance; however, in strongly disturbed areas, the negative impact of alien invaders is less pronounced [14]. Flanagan et al. [15] reported that in contrast to native species, the abundance of which is strongly influenced by climate, for invasive alien species, their abundance is more influenced by site-specific factors, such as land use and soil nutrient availability.

Different invasive alien plant species have different invasion potential. Invasive alien vines can be particularly successful, and they can infect undisturbed riparian zones and spread over native vegetation [10]. Vines can significantly alter the habitat of riparian vegetation and affect the vitality. They are a kind of ‘structural parasite’, as they can alter the habitus of their host plants [16]. Vines increase the density of the crown of woody vegetation, which can become more susceptible to breakage by wind and the weight of snow [15]. Vines compete with their tree hosts for light and space; however, the competitive effects differ according to species [15,17]. Vines also successfully compete for water and nutrients in the soil, because they usually have well-developed root systems and very wide vessels, with many narrow vessels that increase the hydraulic conductivity through the plant itself [16]. The competition can also change over time, due to the developmental cycles of the vines and the host species [18]. They can also have deeper roots compared to the trees [16].

According to their mode of climbing, vines are divided into shoot twiner, tendril climbers, root climbers and scramblers [19]. Many vines show high phenotypic plasticity [20]. Gianoli and Carrasco-Urra [21] reported that the leaves of vines creeping along the ground differ from the leaves of the same species when climbing, while for some vine species, their leaves can mimic those of the host plant, to reduce herbivory pressure. This similarity might prevent the distinction of vines from host plants by remote sensing tools.

The annual herbaceous vine *Echinocystis lobata* and the perennial woody vine *Parthenocissus quinquefolia* are abundant in riparian zones in Slovenia. They show dense growth, and hence can cover the native vegetation [22,23]. The present study examined the effects of these two vines on the selected plant and habitat characteristics of their respective hosts, the willows *Salix fragilis* and *S. caprea*. We also examined how the leaves of the vines and their specific hosts differed in their optical properties, as leaf light reflectance and transmittance can define the plant energy balance [24]. Leaf reflectance is also known as the ‘spectral signature’ of plants, which can serve as a highly valuable tool to define plant vitality, and in some cases it enables the distinction between species [25]. The spectral signatures of the species examined here might serve as a basis for the detection of invasive alien vines in riparian zones by remote sensing.

### 2. Materials and Methods

#### 2.1. Plant Material

The experimental plants in this study were the invasive alien vines wild cucumber (*Echinocystis lobata* (Michx.) Torrey et A. Gray) and Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch), which had spread over their respective host plants of goat willow (*Salix caprea* L.) and crack willow (*S. fragilis* L.). Wild cucumber is a summer annual that belongs to the family of Cucurbitaceae. It has forked tendrils for climbing. The fruits and seeds can spread by falling off, to remain and germinate in the vicinity of the mother plant (i.e., gravitational spread), or by falling into water (i.e., hydrochory spread). Fresh ripe seeds are dormant and have to undergo stratification for successful germination [22]. The Virginia creeper is a perennial, deciduous, climbing woody vine that belongs to the grape family. It is defined as an adhesive-tendril climber [26]. It often occurs in man-made habitats as an ornamental plant, and is also found in riparian habitats and in wood margins. It thrives on nutrient-rich soils, and its seeds can be dispersed over long distances by birds [23].
Both of these vine species are rapidly growing and can climb or trail along the ground. They originated in North America and are now widely spread and highly invasive in Europe [22,27].

Goat willow (S. caprea) is a deciduous shrub or small tree that is native to Europe and western and central Asia. Its leaves are broader in comparison to other willows, and it colonizes riverbanks and drier sites [28]. Crack willow (S. fragilis) is a medium-sized to large deciduous tree with oblong leaves that thrives in riparian habitats and swamps, and is native to Europe and western Asia.

2.2. Site Description

The experiment was performed in the riparian zone of the river Krka (Slovenia) at two separate sites in stands of each vine/willow pairing: wild cucumber/goat willow in Groblje, Novo mesto (45°47′56″ N, 15°08′50″ E); and Virginia creeper/crack willow in Županječko sprežališče, Novo mesto (45°48′07″ N, 15°10′03″ E). The relationships between these invasive alien vines and their host species were studied at 10 locations for each pairing, with 10 willows without vines also included.

The radiation spectra were measured on a clear summer day using a portable spectrometer (Ocean Optics, Inc., Dunedin, FL, USA). The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectralon, Labsphere, North Sutton, NH, USA). Full radiation was defined as that measured about 1 m outside the canopy of the stand, with the radiation within the stand canopy measured at 1 m above the ground and at ~20 cm within the canopy. The air and soil temperatures were measured with an infrared probe, the relative air humidity was recorded using a hygrometer, and the soil humidity was measured using a moisture probe meter (MPM-160-B; ICT International Pty Ltd., Armidale, NSW, Australia). The physical conditions within the different micro-locations with respect to the host plants were monitored close to the leaf surfaces of the vines and willows. These micro-locations were defined for each stand of host willows without and with their respective vines as outside the stand canopy (about 1 m from the canopy), directly above the stand canopy (at the surface of the tree canopy) and 1 m from the ground at a point positioned about 20 cm within the stand canopy. The soil measures and sampling were about 1 m outside of the stand and between the trees within the stand.

2.3. Spectral Analyses

The optical properties of 10 fresh vital leaves of all four species were measured on the day of sampling, along with separate measures of the 10 willow leaves under the vines for each pairing. Leaf reflectance and transmittance were measured from 290 nm to 900 nm using a portable spectrometer (Ocean Optics, Inc., Dunedin, FL, USA), which was fitted with an integrating sphere (ISP-30-6-R; Ocean Optics, Inc., Dunedin, FL, USA) and an optical fiber (QP600-1-SR-BX; Ocean Optics, Inc., Dunedin, FL, USA). The total adaxial reflectance spectra were recorded during illumination of the leaves with a UV-VIS-near infrared (NIR) light source (DH-2000; Ocean Optics, Inc., Dunedin, FL, USA). The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectralon, Labsphere, North Sutton, NH, USA). For measurements of the transmittance spectra, the UV-VIS-NIR light source illuminated the adaxial leaf surface, and the light passed through the sample into the integrating sphere. The spectrometer was calibrated to 100% transmittance with a light beam passing directly into the integrating sphere.

2.4. Morphological and Anatomical Analyses

The leaf morphological and anatomical analyses were performed on the same leaves used for the leaf optical properties. Specific leaf areas (SLAs) were determined as leaf area per unit dry mass (cm$^2$ g$^{-1}$). The thicknesses of the whole leaf, cuticle, epidermis, palisade and spongy mesophyll were measured under a microscope at 100x magnification (CX41; Olympus), which was equipped with a digital camera (XC30; Olympus), with the CellSens software (Olympus, Tokyo, Japan) being used.
2.5. Biochemical Analyses

The leaf biochemical analyses were also conducted on the same leaves that were used for the leaf optical, morphological and anatomical analyses. All of the biochemical analyses were carried out on leaf extracts, using a UV/VIS spectrometer (Lambda 25; Perkin-Elmer, Norwalk, CT, USA). The contents of chlorophyll a, chlorophyll b, and carotenoids were determined using 100% acetone (v/v), according to Lichtenthaler and Buschmann [29,30]. The absorbance levels of the samples were measured at 470, 645 and 662 nm. The contents of the chlorophylls and carotenoids are expressed per leaf area of each sample. The contents of the anthocyanins were determined at 530 nm using a 37% HCl-100% methanol solution (1:99; v/v), as previously described by Drumm and Mohr [31]. The total methanol-soluble UVB-absorbing and UVA-absorbing substances were determined using 37% HCl-distilled water-100% methanol solution (1:20:79; v/v), according to Caldwell [32], with absorbance measured from 280 nm to 319 nm, and from 320 nm to 400 nm, respectively. The relative contents of the anthocyanins and UV-absorbing substances are reported per sample leaf area.

2.6. Statistical Analyses

Normal distributions of the data were evaluated using Shapiro-Wilk tests. Homogeneity of variance from the means was analyzed using Levene’s tests. One-way analysis of variance (ANOVA) followed by Duncan’s post-hoc multiple range tests was used to define the differences between different plant series for each measured parameter. The SPSS Statistics 22.0 package (IBM) was used for the statistical calculations, with significance accepted at $p \leq 0.05$.

Detrended correspondence analysis was used for the exploratory data analysis, with the CANOCO 4.5 program package. Due to the low gradient lengths obtained (<3 SD) [33], namely 0.32 and 0.56, redundancy analysis (RDA) was used to determine whether leaf reflectance and transmittance of the sample plants were related to variations in their leaf morphological and biochemical properties. The significance of the effects of the variables was determined using Monte Carlo tests with 999 permutations. Forward selection of the explanatory variables was used to avoid co-linearity. All of the variables used in the analysis were standardized.

3. Results

3.1. Environmental Conditions

Both of the vines studied altered the radiation environment of their respective willow hosts. Figure 1 shows the reduction of the radiation due to the vines, which occurred especially in the UV-A and visible regions of the spectrum. *P. quinquefolia* was more efficient in reducing the incoming radiation compared to *E. lobata*. Both of these vines showed similar effects on the quality of the light. Within all of the stand canopies, there were shading effects with strong reductions in the radiation in the red and blue regions, while most of the light was transmitted in the green region.

The relative air humidity and temperature were measured for three locations relative to the willows without and overgrown by the vines. These showed that the microclimatic conditions under the vines did not differ significantly from those for the willows growing without vines (Table 1). However, there was a trend to increased relative air humidity under the vines, and reduced air and soil temperatures with *P. quinquefolia*. 
Water 2019, 11, x FOR PEER REVIEW 5 of 13









Figure 1. Relative solar radiation levels at the different wavelengths for the willow canopies. The full radiation was measured about 1 m outside the stand canopy, and the radiation within the stand canopy was measured at 1 m above the ground and at ~20 cm within the canopy, for the stands of S. fragilis without (Salix fragilis) and overgrown by P. quinquefolia (Salix fragilis B), and for those of S. caprea without (Salix caprea) and overgrown by E. lobata (Salix caprea B).

Table 1. Selected physical parameters measured for the willow stands without and overgrown by the two vines. These micro-locations for the air parameters were outside the stand canopy (about 1 m from the canopy), directly above the stand canopy (at the surface of the tree canopy), and 1 m from the ground at a point positioned about 20 cm within the stand canopy. For the soil parameters, they were about 1 m outside of the stand and between the trees within the stand.

| Parameter                  | Units | Stand Location       | S. caprea   | S. fragilis |
|----------------------------|-------|----------------------|-------------|-------------|
| Air temperature (°C)       |       | Without Overgrown a  | 30.8 ± 1.4  | 30.9 ± 1.6  |
|                            |       | Above                | 28.1 ± 1.6  | 29.4 ± 1.5  |
|                            |       | Within               | 25.6 ± 1.2  | 26.4 ± 1.4  |
| Relative air humidity (%)  |       | Outside              | 41.4 ± 2.3  | 44.8 ± 6.4  |
|                            |       | Above                | 45.6 ± 3.2  | 48.5 ± 4.5  |
|                            |       | Within               | 50.1 ± 4.6  | 51.3 ± 3.9  |
| Soil temperature (°C)      |       | Outside              | 31.2 ± 2.9  | 27.3 ± 3.3  |
|                            |       | Within               | 22.2 ± 0.8  | 24.0 ± 1.4  |
| Soil humidity (%)          |       | Outside              | 18.7 ± 6.3  | 24.0 ± 7.4  |
|                            |       | Within               | 19.7 ± 7.0  | 21.6 ± 4.6  |

Data are means ± standard deviation (SD) (n = 10 for each species); ns, non-significant; a, S. caprea overgrown by Echinocehis lobata; b, S. fragilis overgrown by Parthenocissus quinquefolia.

3.2. Leaf Traits

The vine leaves contained less chlorophyll per area than the willow leaves, although E. lobata had the same leaf thickness, while P. quinquefolia had thicker leaves (Table 2). Both of the vines also showed lower contents of UV-absorbing substances than the willows. The vines did not affect the levels of these UV-absorbing substances in the willows. The presence of E. lobata positively affected the content of chlorophylls and carotenoids in its host S. caprea, which was opposite to that seen for P. quinquefolia and its host S. fragilis.
Table 2. Leaf traits of the vines and the willows growing without vines and overgrown with vines.

| Leaf Traits | Units | Echinocystis lobata | Salix caprea | Parthenocissus quinquefolia | Salix fragilis |
|-------------|-------|---------------------|--------------|-----------------------------|--------------|
|             |       | Without Overgrown   |              | Without Overgrown           |              |
| Biochemical |       |                     |              |                             |              |
| Chlorophyll a | mg/cm² | 0.025 ± 0.004 a     | 0.035 ± 0.004 c | 0.046 ± 0.006 b           | 0.019 ± 0.0048 a | 0.044 ± 0.0051 b | 0.035 ± 0.004 c |
| Chlorophyll b | mg/cm² | 0.022 ± 0.006 c     | 0.028 ± 0.006 ac | 0.036 ± 0.006 b           | 0.019 ± 0.005 ac | 0.040 ± 0.008 b | 0.022 ± 0.005 ac |
| Carotenoids  | mg/cm² | 0.006 ± 0.001 b     | 0.008 ± 0.001 a | 0.011 ± 0.002 c           | 0.003 ± 0.001 d | 0.001 ± 0.005 e | 0.008 ± 0.001 a |
| Anthocyanins | RU/cm² | 0.65 ± 0.17 b       | 1.32 ± 0.44 abd | 1.90 ± 0.12 ac            | 0.99 ± 0.53 abc | 0.97 ± 0.25 bc  | 1.57 ± 0.14 d   |
| UV-B-AS      | RU/cm² | 1.88 ± 0.38 b       | 3.86 ± 0.87 a  | 5.18 ± 0.65 a             | 0.95 ± 0.29 c  | 4.27 ± 0.80 a  | 4.45 ± 1.22 a   |
| UVA-AS       | RU/cm² | 2.94 ± 0.56 d       | 7.94 ± 1.34 a  | 6.15 ± 1.13 ab            | 1.18 ± 0.23 d  | 4.31 ± 0.72 c  | 5.14 ± 0.97 bc  |
| Morphological |       |                     |              |                             |              |
| Specific leaf area | cm²/mg | 0.44 ± 0.07 a     | 0.14 ± 0.02 bc | 0.13 ± 0.02 b             | 0.55 ± 0.11 a   | 0.15 ± 0.02 b | 0.18 ± 0.04 c   |
| Upper cuticle | µm    | 4.4 ± 1.1 ab       | 4.5 ± 1.0 ab  | 1.5 ± 0.2 c               | 8.7 ± 1.6 d    | 5.8 ± 1.4 a   | 3.6 ± 0.3 b    |
| Upper epidermis | µm | 14.0 ± 2.6 a      | 15.4 ± 4.2 ab | 8.3 ± 1.0 c               | 15.5 ± 3.7 b   | 12.0 ± 2.8 ab | 14.8 ± 0.9 ab  |
| Lower cuticle | µm    | 3.6 ± 1.3 ac       | 4.3 ± 1.0 a   | 1.2 ± 0.2 d              | 6.3 ± 1.9 b    | 4.7 ± 1.4 ab  | 2.2 ± 0.1 a    |
| Lower epidermis | µm | 8.7 ± 2.8 a       | 11.2 ± 2.6 ab | 9.1 ± 1.1 a              | 16.7 ± 3.6 b   | 8.2 ± 2.7 a   | 12.6 ± 1.8 b   |
| Leaf thickness | µm | 116.3 ± 14.7 a     | 136.7 ± 14.9 ab | 124.6 ± 17.0 a           | 237.9 ± 26.5 c | 141.3 ± 15.6 b | 156.1 ± 14.9 b |
| Leaf tissue density | mg/cm³ | 203 ± 39 b      | 537 ± 110 d   | 618 ± 49 d               | 80 ± 15 a     | 511 ± 163 8   | 398 ± 72 c   |
| Reflectance  | %     | 8.39 ± 1.21 a     | 11.10 ± 1.12 c | 12.70 ± 1.69 b           | 9.23 ± 0.81 a  | 13.20 ± 0.52 b | 10.55 ± 0.44 c  |
| UV-A        | %     | 7.17 ± 1.06 c     | 9.22 ± 0.99 a  | 9.78 ± 1.04 b           | 8.12 ± 0.81 b  | 9.54 ± 0.37 a  | 8.43 ± 0.21 b  |
| Violet      | %     | 4.87 ± 0.57 a     | 4.84 ± 0.37 a  | 6.03 ± 0.50 b           | 4.83 ± 0.35 a  | 7.08 ± 0.22 b  | 6.38 ± 0.11 b  |
| Blue        | %     | 5.06 ± 0.50 a     | 4.65 ± 0.33 b  | 5.91 ± 0.48 c           | 4.67 ± 0.36 ab | 6.70 ± 0.23 d  | 6.10 ± 0.14 c  |
| Green       | %     | 11.45 ± 1.34 d    | 8.54 ± 0.86 ab | 8.21 ± 0.72 a           | 10.06 ± 1.49 b | 9.82 ± 1.01 bc | 8.90 ± 0.52 abc |
| Yellow      | %     | 9.11 ± 1.24 a     | 7.08 ± 0.98 b  | 7.47 ± 0.58 b           | 7.52 ± 1.13 b  | 8.08 ± 0.74 ab | 7.65 ± 0.48 b  |
| Red         | %     | 6.27 ± 0.65 c     | 5.37 ± 0.58 a  | 6.92 ± 0.51 b           | 5.25 ± 0.56 a  | 6.88 ± 0.32 b  | 6.88 ± 0.30 b  |
| Near infrared | % | 34.61 ± 1.79 a    | 33.80 ± 1.84 a | 35.46 ± 4.40 bc         | 35.31 ± 1.35 ab | 44.60 ± 1.60 d | 38.23 ± 2.07 c |
| Transmittance | % | 0.02 ± 0.08 a     | -0.08 ± 0.13 a | 0.14 ± 0.14 a           | 0.04 ± 0.11 a  | -0.10 ± 0.10 a | 0.13 ± 0.10 a  |
| UV-B        | %     | -0.07 ± 0.07 a    | -0.08 ± 0.09 a | 0.12 ± 0.30 a           | -0.04 ± 0.10 a | -0.16 ± 0.12 a | 0.13 ± 0.10 a  |
| UV-A        | %     | 0.91 ± 0.54 abcd  | 0.19 ± 0.18 b  | 0.52 ± 0.89 abc          | 1.99 ± 1.16 d  | 0.29 ± 0.22 ab | 0.86 ± 0.36 cd |
| Blue        | %     | 2.02 ± 1.07 bc    | 0.72 ± 0.39 a  | 0.94 ± 1.27 ac           | 3.38 ± 1.62 b  | 0.92 ± 0.45 ac | 1.71 ± 0.54 bc |
| Green       | %     | 14.35 ± 1.96 c    | 6.34 ± 1.02 a  | 5.70 ± 1.76 a           | 17.59 ± 4.02 d | 7.91 ± 1.65 ab | 10.40 ± 1.30 b |
| Yellow      | %     | 12.28 ± 2.08 a    | 5.36 ± 0.92 b  | 4.90 ± 2.18 b           | 14.54 ± 3.75 a | 6.04 ± 1.49 bc | 8.36 ± 1.23 e  |
| Red         | %     | 6.20 ± 1.70 ab    | 2.56 ± 0.73 c  | 3.14 ± 2.39 c           | 8.37 ± 2.68 a  | 2.84 ± 1.03 c  | 4.57 ± 0.86 bc |
| Near infrared | % | 45.47 ± 3.07 ab   | 36.10 ± 3.44 a | 42.32 ± 4.89 a          | 54.60 ± 3.75 c | 43.14 ± 2.35 ab | 53.94 ± 1.91 c |

Data are means ± SD (n = 10 for each species); 6. Salix caprea overgrown by Echinocystis lobata; 7. Salix fragilis overgrown by Parthenocissus quinquefolia; different superscript letters within each row indicate significant differences (p ≤ 0.05; Duncan tests); reflectance and transmittance spectra represent means within 5-nm intervals (p ≤ 0.05; Duncan tests); RU, relative units; UVB-AS, UVB-absorbing substances; UVA-AS, UVA-absorbing substances.
The vines both shown significantly lower SLA and tissue density than the willows (Table 2). The presence of the vines also negatively affected the upper and lower cuticle thicknesses of the willow leaves, which were significantly thinner for the willow leaves growing under the vines.

The leaf optical properties differed significantly between the vines and the willows in some of the spectral regions (Table 2; Figure 2). The vines reflected less light in the UV region and more light in the green region. The reflectance spectra of the two vines differed significantly in the green, yellow and red regions of the spectrum. The reflectance of the E. lobata leaves differed compared to its host willow in all of the regions, with the exception of violet and NIR, while for P. quinquefolia, there were differences along the whole spectrum, with the exception of the green and yellow regions. The overgrowth by P. quinquefolia of the willow S. fragilis affected the reflectance of this willow species to a similar extent as for E. lobata and S. caprea. This effect was generally less pronounced than the differences between the two willows.

For the transmittance spectra, there were fewer significant differences seen. The leaves of the vines were more transparent to light in comparison to those of the willows, and transmitted more light, especially in the visible part of the spectrum. The most pronounced differences here compared to the willows were in the green, yellow and red regions of the spectrum (Table 2).
3.3. Relationships between Leaf Structural Traits and Leaf Optical Properties

Redundancy analysis was run to determine the relationships between the leaf biochemical traits and the leaf reflectance spectra. This showed that for the variability of the reflectance spectra, chlorophyll a content explained 28% ($p = 0.001$), anthocyanins 9% ($p = 0.001$) and UVB- and UVA-absorbing substances an additional 4% each ($p = 0.01$) (Figure 3). Altogether, as much as 45% of the variability of the reflectance spectra was explained by the biochemical parameters. In the RDA plot, the different samples formed distinct groups, with some overlap for both of the $S. caprea$ groups. In a separate RDA that was run for the morphological parameters, this showed that for the variability of the reflectance spectra, SLA explained 26% ($p = 0.001$), leaf thickness 11% ($p = 0.001$) and upper cuticle an additional 6% ($p = 0.001$), altogether representing 43% of the variance.

Redundancy analysis of the relationships between the leaf biochemical traits and the transmittance spectra showed that for the variability of the transmittance spectra, UVB- and UVA-absorbing substances explained 40% ($p = 0.001$) and 4% ($p = 0.031$), respectively, carotenoids 6% ($p = 0.006$) and anthocyanins an additional 3% ($p = 0.035$) (Figure 4). For the morphological traits, RDA only showed SLA as significant, which explained 51% ($p = 0.001$) of the transmittance spectra variability.

![Figure 3. Redundancy analysis ordination diagram showing the strengths of the associations between the leaf biochemical parameters (i.e., carotenoids, UVA- and UVB-absorbing substances, chlorophyll a and anthocyanins) and the regions of the reflectance spectra for the leaves of the vines and willows. White diamonds, $E. lobata$; white squares, $P. quinquefolia$; white upright triangles, $S. fragilis$; gray upright triangles, $S. fragilis$ shaded by $P. quinquefolia$; white upside-down triangles, $S. caprea$; gray upside-down triangles, $S. caprea$ shaded by $E. lobata$. Only the significant leaf parameters are shown.](image-url)
was rather low, which might be very important for vines, as it reduces the overall mass of the plant, and therefore presents a lesser threat to their host. Mendez-Alonso et al. [39] found out that the mechanical properties of leaves depend strongly on tissue density of the leaf tissue of both vines used for new assimilation areas. In contrast, perennials have a more conservative strategy, saving for safety, as they also invest in storage deposits [38]. However, the density of the leaf tissue of both vines was double that of the annual species

4. Discussion

Invasive alien plants may negatively affect native riparian vegetation richness [34], since they compete with native species for water, nutrients and space. Indirectly, they can also influence aquatic community structure by altering riparian zone productivity and nutrient cycling [35,36]. McNeish et al. [37] reported about significantly higher macroinvertebrate density in reach without the invasive alien shrub Lonicera maackii in comparison to that colonized with this species. In addition to all these effects, invasive alien vines overgrowing the riparian vegetation reduce incoming light for their hosts and increase the density of the canopy crowns, which can then become prone to breakage by wind and the weight of snow [15]. In the present study, the host plants overgrown by the vines received significantly reduced radiation, although there were no significant changes in the soil and air temperatures and humidities.

The responses to these reduced light environments were different for each of the willows, as overgrowth of the willow host Salix caprea by the vine E. lobata positively affected the contents of chlorophylls and carotenoids, which was opposite to the overgrowth of the willow host S. fragilis by the vine P. quinquefolia. The differences in the biochemical responses of willows to vines may be a consequence of the growth dynamics of both vines, which may not be completely the same. To evaluate this aspect, their growth dynamics should also be monitored. These differences might also be related to the leaf traits of the perennial species P. quinquefolia, where the thickness of its leaves was double that of the annual species E. lobata. Annuals are categorized as investors, since they have a high proportion of photosynthetically-active tissues and high photosynthetic capacity, where carbon “income” is mainly used for new assimilation areas. In contrast, perennials have a more conservative strategy, saving for safety, as they also invest in storage deposits [38]. However, the density of the leaf tissue of both vines was rather low, which might be very important for vines, as it reduces the overall mass of the plant, and
therefore presents a lesser threat to their host. Mendez-Alonzo et al. [39] found out that the mechanical properties of leaves depend strongly on tissue density. Vines’ leaves are well supported by their hosts, therefore, lower tissue density was expected according to efficient plant economy. In spite of the good light accessibility for the vines climbing over the willows, *P. quinquefolia* is highly adapted to shade, as it has a low light compensation and saturation point, at <10% of full light, and a relatively high photosynthesis rate [26]. As shown in the present study, the leaves of *P. quinquefolia* have relatively thick upper and lower cuticles, while the leaves of *E. lobata* were the thinnest among these four species, although the density of the leaf tissue was also relatively low. In vines, we also obtained significantly lower amounts of methanol-soluble UV-absorbing substances compared to willows. These substances protect plants against high UV radiation levels [40,41], and may also significantly affect the litter decomposition rate [42], and thus cycling of matter in aquatic ecosystems. Lower amounts of these substances are possibly related to quick growth of vines [22,27], as resistance against environmental constraints is often not compatible with fast growth [43]. Increased plant growth might be accompanied by down-regulation of different plant defense genes [44], including those for most of the categories of secondary metabolites.

Both of these willows growing under the vines had significantly thinner cuticles than those growing without the vines, which may also be an important factor affecting the litter decomposition process [45]. The leaf cuticle is an extracellular hydrophobic layer that covers the epidermis of land plants, to prevent their desiccation and to mitigate the effects of different external environment stressors [46], including high light intensity [38]. The cuticle is synthesized by the epidermal cells, which respond to developmental and environmental triggers [47]. Thus, the thinner cuticles in the willows growing under the vines might lead to a higher vulnerability of these leaves to abiotic stressors, pests and pathogens, as the cuticle represents the first barrier at the leaf surface [46].

The pronounced differences in the leaf morphological and biochemical traits between the vines and their hosts therefore suggest significant differences in the quality of plant litter that will enter the river, which may affect riparian and aquatic food webs, as shown by many studies [48-50]. In addition, vigorous growth of the vines influences the uptake of nutrients from the riparian zones.

This will also change the dynamics of the other primary producers in these riparian zones, as well as the river diatom communities, which are very sensitive to changes in nutrient concentrations [51]. Invasive alien plant species have specific phenology that may lead to changes in the timing of allochthonous organic matter input into aquatic ecosystems [52].

Light transmitted through the canopy enables undisturbed photosynthesis in the assimilation areas under the canopy surface. The amounts of blue and red light that are associated with the leaf characteristics and that develop under high irradiance are of great importance [53]. Transmittance spectra can show considerably more variations in the visible region than reflectance spectra [54]. This was also the case in the present study, where the leaves of the vines were more transparent to blue and red light, which represents an advantage for their hosts. Here, blue light is required for normal photosynthetic functioning and quantitatively mediates leaf responses to irradiance intensity. Furthermore, physiological disorders associated with leaf development under red light alone can be eliminated by the addition of only a small amount of blue light [53]. The *S. fragilis* overgrown by *P. quinquefolia* showed higher transmittance in the visible regions. This was possibly related to the higher SLA and lower leaf tissue density of the vines [54]. The leaf optical properties in the species examined here were explained by the leaf biochemical and morphological traits, among which chlorophyll a appears to be the most influential for explaining reflectance, while SLA explained more than half of the variability of the transmittance spectra.

Due to the multiple negative effects of invasive alien plant species on native biota, the detection of invasive alien species using their optical properties has been studied intensively [55]. However, for vines, it is important that their optical properties differ significantly from their host plants. In general, assessment of non-native species is not fully integrated into most schemes, although the presence of invasive alien species means that infected reaches will not meet high ecological status [51]. The present
study has revealed that the reflectance of the vine leaves showed the greatest differences to the reflectance of the willow leaves in the UV, red and NIR regions, while the leaf transmittance showed differences along the entire visible region. The reflectance and transmittance of light in these regions were higher in the vines than the willows. The only difference between the two vines here was for the transmittance in the green region. These data show that vines can be discerned from their hosts using their spectral signatures.

5. Conclusions

The vines altered the environments of both of their willow host species in terms of the quality and quantity of the radiation. The vines had significantly lower leaf tissue density, which represents an advantage for their host plants from the point of view of weight and light penetration, and might affect the quality of the leaf litter that enters the river. The willows overgrown by the vines had significantly thinner cuticles in comparison to those growing without vines, which might increase the vulnerability of these willows to different biotic and abiotic stressors, and thus might affect the overall function of such riparian zones. These data on the leaf optical properties reveal that while these vines can be discerned from their willow hosts, the spectral signatures between the vines differ only in the green region of the spectrum.

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