Abstract: The holoparasite/host interaction of Pilostyles inae (Rafflesiaceae) and Mimosa naguirei (Mimosaceae) was studied in the open campo rupestre vegetation of Serra do Cipó (State of Minas Gerais, Brazil). Infected M. naguirei plants found at this site were densely covered by flowers of the parasite on their stems indicating heavy development of cellular threads of the parasite in the bark of the hosts. Cellular threads of the parasite are likely to be richer in lipids and hence depleted in $^{13}$C. This may explain the significantly more negative carbon isotope ratios ($\delta^{13}$C values) of the bark of infected host plants observed as compared to other tissues of infected and non-infected host plants. Photosynthetic parameters such as potential quantum yield of photosystem II ($F_v/F_m$), apparent photosynthetic electron transport rates (ETR) and effective quantum yield of photosystem II ($\Delta F/F_m$) in light dependence curves, as well as $\delta^{13}$C values of leaves as a relative measure of average intercellular CO$_2$ partial pressure during photosynthesis over the lifetime of the leaves, which is also related to average stomatal conductance via water use efficiency, were remarkably similar. This suggests a well balanced relation between the Mimosa host and the Pilostyles parasite, in contrast to other hemiparasitic angiosperm parasite/host interactions where the parasite (e.g. Striga) is known to have strong detrimental effects on host photosynthesis.

Key words: Brazil, carbon isotope ratio, chlorophyll fluorescence, holoparasite, Rafflesiaceae.

Abbreviations:
ETR apparent photosynthetic electron transport rate
$F_v/F_m$ potential quantum yield of PS II
$\Delta F/F_m$ effective quantum yield of PS II
PPFD photosynthetic photon flux density
PS photosystem

Introduction

Like all species of the family Rafflesiaceae the members of the genus Pilostyles are holoparasites. Their vegetative organs are reduced to ramifying cellular threads penetrating the tissues of the host plants (Kummerow, 1962; Dell et al., 1982; Kuijt et al., 1985). In the case of Pilostyles species, which parasitize the aerial parts of their host plants, flowers develop on adventitious shoots on the "mycelium" at the external surface of their hosts (Fig. 1). The diameter of the flowers is about 1 mm. Pilostyles species are unisexual dioecious plants feeding on Leguminosae. They are univorous, i.e. very host specific with each species having only one given host (Ule, 1915). They show an unusual geographic distribution: S USA to tropical S America; tropical Africa; Iran; W Australia (Willis, 1973).

In the campo rupestre at Serra do Cipó, Minas Gerais, Brazil, a large number of individuals of Mimosa naguirei Barneby (Mimosaceae) are parasitized by Pilostyles inae (Karst.) Hook. f. The parasite influences the architecture of the host plant: parasitized plants have an increased number of branches, which are shorter than branches on non-parasitized individuals. The number of fruits produced by M. naguirei is not influenced by the parasite, but the fruits are smaller and have lighter seeds.

In the present study we wanted to find out if and to what extent the heavy development of the parasite possibly impedes host photosynthesis, and therefore checked some ecophysiological aspects of this host/parasite interaction. Carbon isotope ratios ($\delta^{13}$C values) were determined as an indication of chemical composition and/or average intercellular CO$_2$ concentration during photosynthesis over the whole life period of leaves as influenced by stomatal conductance and water supply. In leaves of healthy and infected host plants measurements of potential quantum yield of photosystem II were performed, and light dependence curves of apparent photosynthetic electron transport rates and effective quantum yield of PS II were obtained to characterize the photosynthetic apparatus.

* Dedicated to Prof. Dr. Rainer Kollmann on the occasion of his 65th birthday.
Materials and Methods

Study site and plant material

The field studies (chlorophyll fluorescence measurements) were carried out at Serra do Cipó in the central part of the state of Minas Gerais, Brazil (19° 12' S, 43° 28' W) in October 1995. A detailed description of the site is given by Gomes and Fernandes (1994).

Potential quantum yield of photosystem II (Fv/Fm) was obtained after 30 min dark adaptation of individual pinnules of the Mimosa leaves using a pulse-amplitude modulated photosynthesis yield analyzer (Mini-PAM, H. Walz, Effeltrich, Germany). Due to overcast cloudy weather on the day of the measurements, ambient photosynthetic photon flux density (PPFD) was only 380 μmol m⁻² s⁻¹ (λ = 400–700 nm). Therefore, photosynthetic capacity of leaves of non-infected and infected Mimosa plants was checked using the light curve programme of the instrument. Actinic light on the leaves was increased up to ca. 2000 μmol m⁻² s⁻¹ during 4 min in 8 steps following each other within 30 s. At each level of actinic light supplied by the instrument a pulse of saturating irradiance (ca. 6000 μmol m⁻² s⁻¹) was applied to obtain chlorophyll fluorescence parameters. The effective quantum yield of PS II is given as ΔF/Fmₘ = (Fm' - F)/Fm where F is chlorophyll fluorescence of the light-adapted sample and Fm is the maximum light-adapted fluorescence during a saturating light pulse. Apparent photosynthetic electron transport rates (ETR) are given as 0.5 × ΔF/Fmₘ × PPFD (Schreiber and Bilger, 1993), where the factor 0.5 accounts for excitation of both PS II and PS I. No correction was made for reflection since this was not known numerically for the leaves of M. naguirei but must have been similar for all leaves measured. Due to the rapid increase in actinic light intensity over only 4 min, photosynthesis of the leaves was not in steady state at any time during these measurements. Hence, steady state values of ETR were not obtained. On the other hand, the method allows rapid comparisons under closely comparable conditions in the field. In fact, it was our aim mainly to compare the comportment of leaves of non-infected and infected plants. Since, except for the infection, all conditions were strictly comparable as non-infected and infected plants grew in close proximity at the same site, such a comparative assessment is warranted.

The carbon isotope ratios (δ¹³C values relative to Pee Dee belemnite standard) were determined according to Osmond et al. (1975) using a Heraeus CHN rapid elemental analyser coupled on-line to a trapping box gas isotope mass spectrometer system (Finnigan MAT Delta Sx).

Table 1. δ¹³C values (‰) of tissues of non-infected and infected hosts (M. naguirei) and female flower buds of the parasite (P. ingae). Values are x ± SD (n). For the comparisons latin letters (first letters) only refer to comparisons within vertical columns, i.e. to comparisons of the various tissues of non-infected and infected plants, while greek letters (second letters) only refer to comparisons in horizontal lines, i.e. between non-infected and infected plants for a given tissue. Values followed by different letters are statistically significantly different at the p < 0.05 level and for β* at the p < 0.001 level (Mather’s t-test).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>non-infected</th>
<th>infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf</td>
<td>-28.99 ± 0.65 (7) a, α</td>
<td>-29.61 ± 0.23 (3) a, β</td>
</tr>
<tr>
<td>bark</td>
<td>-28.33 ± 0.39 (4) b, α</td>
<td>-30.33 ± 0.39 (3) b, β*</td>
</tr>
<tr>
<td>wood</td>
<td>-28.33 ± 0.45 (4) b, α</td>
<td>-28.58 ± 0.83 (3) a, α</td>
</tr>
<tr>
<td>female flower buds</td>
<td>-28.36 ± 0.28 (2) a</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Female flower buds of the holoparasite Pilostyles ingae on stems of the host Mimosa naguirei (Serra do Cipó, Minas Gerais, Brazil).
Results and Discussion

Among C₃ plants carbon isotope ratios (δ¹³C values) of the biomass are partly determined by chemical composition, in particular when different types of tissues are compared, and partly by stomatal control, e.g. when leaves are compared as photosynthetic organs. It is well-known, for example, that pyruvate dehydrogenase discriminates against the heavy (¹³C rich) pyruvate, and hence, is responsible for reduction of ¹³C levels in fatty acids (Deniro and Epstein, 1977). Thus, the slightly more negative δ¹³C values (less ¹³C) of leaves of non-infected M. naguirei plants as compared to the bark and the wood (statistically significant at the 0.05 level, Table 1) may be due to the leaves containing more lipids due to the presence of chloroplasts. In the infected M. naguirei plants there are no statistically significant differences between the δ¹³C values of leaves and wood. Conversely, the bark of infected plants has more negative δ¹³C values (i.e. a smaller proportion of ¹³C) than leaves and wood of infected plants (significant at the 0.05 level) and more negative δ¹³C values than the bark (significant at the 0.001 level) and other parts of healthy M. naguirei. It is very probable that the clear difference between δ¹³C values of the bark of infected and non-infected plants mirrors the strong development of the parasite, which is documented by the dense coverage of the stems of infected plants by flowers of the P. ingae parasite (Fig. 1) which must be supported by a vigorous growth of hyphae-like threads of the parasite. These threads are known to be restricted to the infected bark and to be rich in cytoplast, membranes and lipids (Kummerow, 1962). The δ¹³C value of the female flower buds was -28.36±0.28 (2). It did not differ from leaf and wood of the infected host plants and was less negative than the bark of these plants. The flower buds have woody scales and stalks and must have much less lipid than the "hyphae" in the bark of the host.

The light-dependence curves of apparent photosynthetic 
electron transport rates (ETR) and of effective PS II quantum yield (Δ F/Fₘₐ) obtained for leaves of healthy and parasitized plants of M. naguirei, to our surprise, were practically identical (Fig. 2). The speed at which PPFD was increased with the light curve programme of the Mini-PAM was too fast to reach steady state (see Materials and Methods). Thus, these measurements do not rule out that steady photosynthesis might indeed be different between the leaves of infected and non-infected plants. However, they do show that the speed at which the light reactions of photosynthesis are induced during PPFD increase are similar in the two cases. At the ambient PPFD of about 380 μmol m⁻² s⁻¹, given, neither the parasitized nor the healthy plants of M. naguirei were subject to photo-inhibition, since potential quantum yield (Fᵥ/Fₘ) in both cases was close to 0.8 (inset numbers in Fig. 2; see Björkman and Demmig, 1987). The δ¹³C values of leaves of healthy and parasitized plants of M. naguirei also do not differ (Table 1). In C₃ plants carbon isotope ratios indicate average stomatal conductance and intercellular CO₂ concentration during photosynthesis over the whole lifetime of the leaves sampled, which is also related to average stomatal conductance via water use efficiency (Farquhar et al., 1989).

The conformity in key parameters of photosynthetic electron transport and stomatal control in parasitized and healthy host plants may be an indication of a well-balanced relation between host and parasite, perhaps the result of a relatively old phylogenetical interaction. From a teleological point of view, considering a possible advantage in evolution, this is an optimal situation: the heterotrophic parasite does not impair the food-producing process of the host on which it depends.

It would be interesting to compare the influence of other parasitic angiosperms on the photosynthesis of their host
With the holoparasite, Cuscuta reflexa, it was observed that it may have enhancing effects by creating a sink stimulation which is regulated by supply and assimilation of nitrogen (Jeschke and Hilpert, 1997; Jeschke et al., 1997). The present observations did not suggest any detrimental effects of the P. ingae holoparasite infection on photosynthesis of M. naguirei in the field-grown host/parasite consortium.

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References


