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Short communication

Localised photosynthetic pigments in the node of a holoparasitic plant: support for shoot growth?

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ABSTRACT

Understanding the role of photosynthesis in the holoparasitic genus *Cuscuta* may give insights into the biology and evolution of these plants. We hypothesised that light irradiance on the node of *C. racemosa* is important for sustaining the growth of the new shoot through photosynthesis. We blocked light irradiance on the node of detached twigs of *C. racemosa* for one week and measured twig fresh and dry weight, shoot length and photosynthetic pigment content. Even though fresh and dry weight did not differ between non-irradiated and irradiated twigs, the shoots of non-irradiated nodes were significantly shorter. The non-irradiated plants showed higher amounts of chlorophyll *a*, *b*, and lycopene, and a lower concentration of β-carotene. Furthermore, the chlorophyll *a*/*b* ratio was lower than that of the control (irradiated) plants. Beyond corroborating our hypothesis, our results suggest that, when light is blocked on the node of *C. racemosa*, the holoparasite responds in a way similar to that of autotrophic plants under shaded conditions. Our findings suggest a new and previously unacknowledged role for the node of *C. racemosa*, which would be supporting shoot elongation through photosynthesis.

Keywords: β-carotene, carotenoids, chlorophyll, chlorophyll *a*/*b* ratio, *Cuscuta racemosa*, lycopene, plant physiology, photosynthesis

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Introduction

Dodders (Convolvulaceae: *Cuscuta* spp.) are holoparasitic plants which occur in all the continents, except Antarctica (Costea & Stefanović 2010). Typically, they resemble a mass of yellow or orange stems with, at most, minute vestigial scale-like leaves, growing on their plant hosts (Hibberd *et al*. 1998). They do not have roots, and they absorb all their nutrients through haustoria, which are connected to the hosts (Birschwilks *et al*. 2006; Costea & Tardif 2006). Dodders are some of the most devastating agricultural parasites worldwide; however, they are also ecologically relevant owing to the strong effect of their parasitism on the fitness of wild plants and invasive species (Costea & Tardif 2006; Albert *et al*. 2008; Mishra 2009; Cruz Neto *et al*. 2017; Wu *et al*. 2019).

Some dodder species exhibit extremely limited photosynthetic ability, and they cannot live autotrophically (Machado & Zetsche 1990; Hibberd *et al*. 1998). However, under certain conditions, they can synthesise high amounts of chlorophylls (Walzel 1952; Laudi 1968). Indeed, these species harbour many functional photosynthetic plastidial genes and exhibit Rubisco activity, functional electron transport in the photosystems, and CO_2 fixation (MacLeod 1961; Ciferri & Poma 1962; 1963; Pattee *et al*. 1965; Baccarini 1966; Machado & Zetsche 1990; Hibberd *et al*. 1998; McNeal *et al*. 2007). This limited photosynthetic activity is likely triggered during the pre-attachment stage of the seedlings or when the plant is malnourished, such as when detached from its host or growing on a low-quality host (Peirce 1894; Pizzolongo 1963; Pattee *et al*. 1965; Laudi 1968; Lyshede 1985). In a previous study (Parise et al. 2021), we observed that a twig of *Cuscuta racemosa* Mart. detached from the original plant (and from its host) accumulated chlorophyll, particularly in the nodal region (Fig. 1), and the node became greener than the rest of the stem within a few days.

To the best of our knowledge, however, there has been no previous report of chlorophyll accumulation in *C. racemosa*, and the physiology of this plant remains largely unknown. The high photosynthetic pigment content in the node may be important for sustaining the growth of the new shoot while searching for a suitable host. Moreover, by enriching this metabolically active region with pigments, photosynthesis and other photochemical activities may be facilitated at the regions from which the new shoot sprouts. Shoot growth would directly benefit from the products of these photochemical activities through either the Calvin cycle or other. McNeal *et al*. (2007) suggested that dodders could possess an alternative pathway for lipid synthesis which requires a non-photosynthetic activity of Rubisco and could justify the seemingly obsolete photosynthetic apparatus in the genus. This pathway is more efficient in carbon use than the glycolytic pathway, and its efficiency improves with increasing light irradiance. Nevertheless, the photochemical phase of photosynthesis is needed to supply this pathway with ATP and NADPH (Schwender *et al*. 2004).

Figure 1. Node of a detached *Cuscuta racemosa* enriched with green pigments. The white bar represents 0.5 cm. The whole figure had its colour balance adjusted for evidencing colder shades.

Regardless of the mechanisms through which *C. racemosa* accumulates pigments at the node, light and photosynthetic pigments seem highly physiologically relevant. Thus, we hypothesised that blocking light irradiance to the node would impair bud development, because the plant would not be able to harvest light properly. Additionally, since plants in low-light environments present high chlorophylls content to augment photosynthetic efficiency (Lüttge 2008; Souza *et al*. 2009; Ferreira *et al*. 2012), we hypothesised that blocking light irradiance to the node would increase the chlorophyll *a* and chlorophyll *b* content in the plants. Meanwhile, since dodders generally present a high carotenoid content (Baccarini *et al*. 1965; Dinelli *et al*. 1993; Villa-Ruano *et al*. 2017), we expected no significant change in the content of lycopene and β-carotene, which are important carotenoids for plants, because of the blocking of light irradiance to the node.

Materials and methods

The present study was conducted at the campus of Capão do Leão of the Federal University of Pelotas (31°48’06’’ S, 52°25’02’’ W), Rio Grande do Sul, Brazil. During the spring of 2019, a twig of *Cuscuta racemosa* Mart. was collected from an ornamental plant in central Pelotas, Rio Grande do Sul, Brazil, and cultivated on basil plants (*Ocimum* sp.) at the campus in a greenhouse at 28.5 ± 12.9 °C under natural sunlight. When the dodders grew large enough to cover all the hosts, they were used as the stock of *C. racemosa* in the present experiment. The host plants were irrigated twice a week with approximately 200 mL of Hoagland and Arnon solution (Hoagland & Arnon 1950). After allowing to grow for 3 months, the dodders were used in the experiment. Twigs were collected from the stock and trimmed to a length of 10 cm, with only one node at the apical end. All shoots sprouting from the node were excised, leaving only a single bud. All twigs weighed approximately 0.118 ± 0.006 g.

We used six polystyrene boxes (dimensions 20.0 × 25.0 × 17.0 cm), three of each for the experimental and control treatments. A polystyrene shelf (12.0 × 17.0 cm) was installed at a depth of 5.0 cm in each box, and four twigs were placed on the shelves parallel to one another and to the longer side of the box. This arrangement ensured homogenous light irradiance to all twigs. The boxes were then covered with transparent plastic films to maintain humidity.

In the experimental group, light to the node was blocked by wrapping it with a 0.3-cm-wide strip of aluminium foil. In the control group, a similar aluminium foil strip was used to wrap the stem immediately below the node to maintain the effect of the aluminium foil while allowing the node to be illuminated. The boxes containing the twigs were incubated in the growing room of the Laboratório de Cognição e Eletrofisiologia Vegetal (LACEV) at the Universidade Federal de Pelotas at a constant temperature of 25.0 ± 2 °C under a photoperiod of 12.0 h. The plants were placed 50.0 cm below the row of four LED lightbulbs of 50.0 W and approximately 5,000 lumens, with each emitting white light. After 1 week, the dodders were removed from the boxes and analysed.

The initial fresh weight was measured before the treatment, and the final fresh weight and the length of the new shoot were measured after the treatment. From each box, two dodder twigs were randomly selected for biochemical analyses and two for measuring the dry weight.

The content of chlorophyll *a*, chlorophyll *b*, lycopene, and β-carotene were measured using spectrophotometry according to the protocol described by Nagata and Yamashita (1992) with slight modification. Briefly, whole twigs were macerated in hexane:acetone (2:3) and vortexed for 30.0 s. Absorbance of the supernatant was measured using a spectrophotometer (Utrospec® 7000, Biochrom®, Cambridge, UK). The results were expressed in mg 100 g^{-1} of fresh weight. With the content of chlorophyll *a* and *b*, we calculated the chlorophyll *a*/*b* ratio. To determine dry weight, the twigs were placed in a dryer at 44.0 ± 1 °C for 1 week and weighed using a semi-analytical balance (Marte®, model BL-320H, Santa Rita do Sapucaí, MG, Brazil).

Each treatment was performed with three replicates, totalling 12 observations per treatment (four twigs per box). The experimental design was completely randomised, and the data were analysed using ANOVA. When the *F*-values were significant, the treatments were compared using Student's *t*-test ($p \leq 0.05$).

Results

The results of shoot growth and fresh and dry weight are shown in Table 1. The results for biochemical analyses are shown in Table 2. There was a significant difference ($p \leq 0.05$) in shoot length between the two groups, with longer shoots in the control group. Chlorophyll *a*, chlorophyll *b*, and lycopene content was higher in the treatment group, whereas β-carotene content was higher in the control group.

Table 1. Fresh initial weight (FIW), fresh final weight (FFW), dry weight (DW) and shoot length (SL) of dodder twigs with and without aluminium foil involving the node. Values represent the mean + SD (n = 12). Means followed by * showed significant difference ($p \leq 0.05$) by Student's *t*-test.

	Control	Test
FIW (g)	0.113 ± 0.004	0.116 ± 0.006
FFW (g)	0.078 ± 0.009	0.078 ± 0.011
DW (g)	0.008 ± 0.001	0.007 ± 0.001
SL (cm)	12.952 ± 0.447*	11.559 ± 0.482

Table 2. Content of chlorophyll *a*, chlorophyll *b*, lycopene and β-carotene in the dodders with and without aluminium foil involving the node. Values represent mean + SD (n = 12). Means followed by * showed significant difference difference ($p \leq 0.05$) by Student's *t*-test. Values expressed in mg 100 g^{-1} of fresh weight.

	Control	Test
Chlorophyll *a*	4.030 ± 0.562	5.635 ± 0.483*
Chlorophyll *b*	2.436 ± 0.198	4.092 ± 0.615*
Lycopene	0.791 ± 0.067	1.107 ± 0.130*
β-carotene	7.813 ± 0.402*	7.112 ± 0.136

Discussion

Our results showed that the shoot length of *C. racemosa* was significantly shortened when light irradiance to the node was blocked, suggesting that light, specifically at this region, is important for shoot growth. Other growth parameters, however, did not show a significant difference, which indicates the possibility that this result was due to some impairment on elongation mechanisms such as, *e.g.*, the acid growth of the cells. Moreover, the higher chlorophyll content observed in the experimental group corroborated our hypothesis that blocking light irradiance to the node would increase chlorophyll content. However, the increase in lycopene content in the experimental group was unexpected.

Notably, the chlorophyll *a*/*b* ratio was different between the experimental and control groups (1.4 and 1.7, respectively). Blocking of light irradiance to the node increased chlorophyll *b* content by 68 % compared with the control value. Thus, despite the increase in chlorophyll *a* content, the chlorophyll *a/b* ratio was lower in the experimental group than in the control group. Such increases in total chlorophyll content and chlorophyll *a/b* ratio are important for plant acclimation to low-light environments and have been observed in many autotrophic taxa (Lüttge 2008; Souza *et al*. 2009; Ferreira *et al*. 2012; Agathokleous *et al*. 2020). Chlorophyll *a* and *b* absorb light energy at different wavelengths, thus improving the capture of photons required for the photochemical steps of photosynthesis. With slightly different absorption properties, both chlorophyll *a* and *b* enhance the performance of the antenna complexes. For this, the specific binding sites of these complexes must be occupied by either chlorophyll *a* or chlorophyll *b* (Souza *et al*. 2009; Palm *et al*. 2018). Therefore, we suggest that, in the experimental group, the twigs of *C. racemosa* were enhancing its light-harvesting efficiency in the node by synthesising more photosynthetic pigments to increase the chances of catching photons in the node, probably, to maximise the photon capture at the nodes and compensate for the lack of illumination on this part of the plant. Of note, based on the analysed parameters, *C. racemosa* responded to low-light conditions in the same manner as photoautotrophic plants.

Given this result, at first glance, it could be hypothesised that the dodder, by refraining its growth, would be avoiding the shade cast by the aluminium foil. However, the literature available suggests that dodders present the opposite behaviour and have positive tropism towards sources of shade—when it is enriched with far-red radiation—, which is indicative of photosynthetic plants nearby (Orr *et al*. 1996; Benvenuti *et al*. 2005). Additionally, only the node was covered in the experimental group and an equal portion of the internode was covered in the control group. Therefore, plants in both groups received the same amount of light. In conclusion, it is unlikely that the observed behaviour was caused due to shade avoidance.

A higher β-carotene content was observed in the control group than in the experimental group, supporting the photoprotective function of carotenoids in vascular plants (Sun *et al*. 2018). β-Carotene and several xanthophylls (e.g. lutein, zeaxanthin, and violaxanthin) are the constituents of functional multiprotein complexes, such as photosystems I and II, cytochrome b_6/f complexes, and light-harvesting complexes. The protein components of these structures are surrounded and/or bound together by carotenoids through hydrophobic interactions (Domonkos *et al*. 2013). They also participate in the xanthophyll cycle, which is associated with non-photochemical quenching—an energy dissipation mechanism.

Nonetheless, the observed increase in β-carotene content may not necessarily be related to the photoprotection of the photosynthetic apparatus alone, as carotenoids play many other roles in plants such as free radical scavenging, phytohormone synthesis (*e.g.*, ABA), flower and fruit colouration, and membrane protection from lipid peroxidation in the entire cell and not just in the chloroplast (Zakynthinos & Varzakas 2016; Rodriguez-Concepcion *et al*. 2018).

In plant cells, carotenoids are biosynthesised and stored in the plastids, with chloroplasts and chromoplasts showing a higher carotenoid content than other plastids (Sun *et al*. 2018). In a previous carotenoid profiling study, Villa-Ruano *et al*. (2017) reported higher β-carotene and lycopene content in *C. mitraeformis* Engelm. ex Hemsl. than the content noted in the present study in *C. racemosa*. The higher β-carotene content in *C. mitraeformis* (77.4 ± 5.7 mg per 100 g of fresh weight) may be explained by the differences in species, environmental conditions, and carotenoid extraction protocols.

Conversely, blocking of light irradiance at the node increased the lycopene content of dodders. Lycopene is an intermediate of β-carotene in its biosynthesis, and its increase may be related to the decrease of β-carotene. The absence of light at the node of *C. racemosa* might have affected the expression of enzymatic genes or the activity of enzymes involved in various biosynthetic steps, thereby decreasing β-carotene content and leading to lycopene accumulation.

Carotenoid biosynthesis begins with the formation of isopentenyl diphosphate and dimethylallyl diphosphate via the methylerythritol 4-phosphate pathway. In the next step, phytoene synthase catalyses the condensation of two geranylgeranyl diphosphate molecules into phytoene. Finally, lycopene cyclisation yields the ɑ-carotene and β-carotene branches of the pathway, the latter being catalysed by lycopene β-cyclase.

In conclusion, under limited resources due to the unavailability of a host, *C. racemosa* increased photosynthetic pigment content, presumably at the expense of shoot growth, which might explain the smaller shoots in the experimental group than in the control group of this study. Further studies will be needed to verify where the dodders synthesised the higher amounts of chlorophylls. Was it in the illuminated parts of the shoot to compensate for the lack of light at the node, or just in the node to enhance light harvest there? It will increase the knowledge on the importance of nodes and photosynthesis to the shoot growth of *C. racemosa*. Our results raise questions regarding the precise role of photosynthesis and photosynthetic pigments in this holoparasitic plant as well as the importance of nodes in this process.

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