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Allelopathy effects of aqueous rinses of *Dittrichia viscosa* (L.) on the photosynthesis and cell proliferation of N_2-fixing soil cyanobacteria

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Introduction

Allelopathy is a phenomenon in which plant exudates cause chemical effects on other plants and microorganisms (Inderjit and Keating 1999). *Dittrichia viscosa* (L.) W. Greuter (syn. *Inula viscosa* (L.) Aiton) (*Asteraceae*) is an evergreen shrub, widespread in the Mediterranean region. Epicuticular rinses from leaves of *D. viscosa* showed strong allelopathic effects against plants (Stephanou and Manetas, 1995). Rain rinses of *D. viscosa* end up in the soil. N_2-fixing soil cyanobacteria perform higher plant-like oxygenic photosynthesis involved in N_2-fixation using specialized cells the hetrocysts. Heterocysts are specialized cells of filamentous cyanobacteria, in which PS II and photosynthetic O_2 evolution are inactivated, but which contribute to the energetic balance of the filament via PS I-dependent cyclic electron transport, and via N_2 fixation. In the present study we investigated the allelopathy effects of the epicuticular rinses from *D. viscosa* on growth, photosynthetic activity, respiration and heterosyst formation of the N_2-fixing soil cyanobacteria *Nostoc sp*. PCC 7413 and *Anabaena sp.* PCC 6309.

Materials and methods

Plant shoots were harvested during summer, which is the period of maximal concentration of epicuticular flavonoid materials on the leaves. Fresh shoots corresponding to 10 g of dry mass were immersed in distilled water (100 ml) for 3 h with gentle shaking. The resulting solution was passed through a 0.45 μm pore filter to remove foreign objects (dust, fungal spores etc.) and was freeze-dried. The dried material was re-dissolved in BG 11_0 and was used for bioassays. N_2-fixing soil cyanobacteria were cultured at 31 C^0 with white fluorescent light (100 μmoles m^{-2} s^{-1}). Cells were grown in $BG11_0$ (Rippka et al 1979) which was buffered with 20 mM Hepes-NaOH pH 7.5. Culture growth was measured in the terms of turbidity (A_{730}) and Chl a was measured according to Moran (1982). Photosynthetic oxygen evolution ($H_2O\rightarrow CO_2$), photosystem (PS) II activity ($H_2O\rightarrow$ phenyl-p-benzoquinone (PBQ)), photosynthetic electron transport ($H_2O\rightarrow$ methyl-viologen (MV)) and dark respiration were measured in a Clark-type oxygen electrode. The number of heterocysts was quantitated using a hematocytometer.

Results and Discussion

We examined the effects of epicuticular material from leaves of *D. viscosa* on growth of N_2-fixing soil cyanobacteria *Nostoc* sp. PCC 7413 and *Anabaena* sp. PCC 6309. Epicuticular material inhibited cell proliferation of *Nostoc* sp. PCC 7413 in a dose-related manner (Fig. 1).

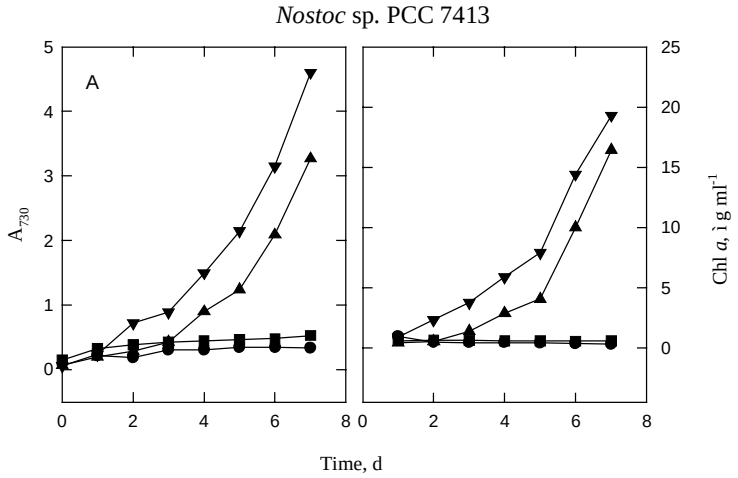

Fig. 1. Culture growth expressed in terms of turbidity A_{730} (A) and Chl *a* concentration (B) of N_2-fixing soil cyanobacterium *Nostoc* sp. PCC 7413 in the absence (▼) and in the presence of 0.2 mg (▲), 0.4 mg (■), 0.8 mg (●) epicuticular material/ml culture medium

Fig. 1A shows that in the presence of 0.2 mg epicuticular material/ml culture medium proliferation of *Nostoc* cells was delayed compared to control cells, while in the presence of 0.4 mg and 0.8 mg epicuticular material/ml culture medium, *Nostoc* cells did not grow at all. The same effects were observed also with regard to the Chl *a* content per unit volume of the cyanobacterial culture (Fig. 1B). The second cyanobacterium we tested, *Anabaena* sp. PCC 6309, gave similar results (Fig. 2).

To analyse the causes of the observed inhibitions in cell proliferation, we investigated the effects of extracted epicuticular material from *D. viscosa* on photosynthetic electron transport activities by monitoring light-induced oxygen evolution, or light-induced oxygen uptake of the cyanobacterial cell suspensions. After incubation of cyanobacterial cells with 0.8 mg/ml epicuticular material, the rate of photoinduced oxygen evolution with CO_2 as terminal electron acceptor (total photosynthesis) declined to 30% of the original rate, while the rate of photoinduced electron transport from water to MV (through both PSII and PSI) declined 5% of the original rate. When PBQ was used as electron acceptor, in order to measure the PS II activity alone, there was no inhibition by 0.8 mg/ml epicuticular material in the assay medium (Table 1).

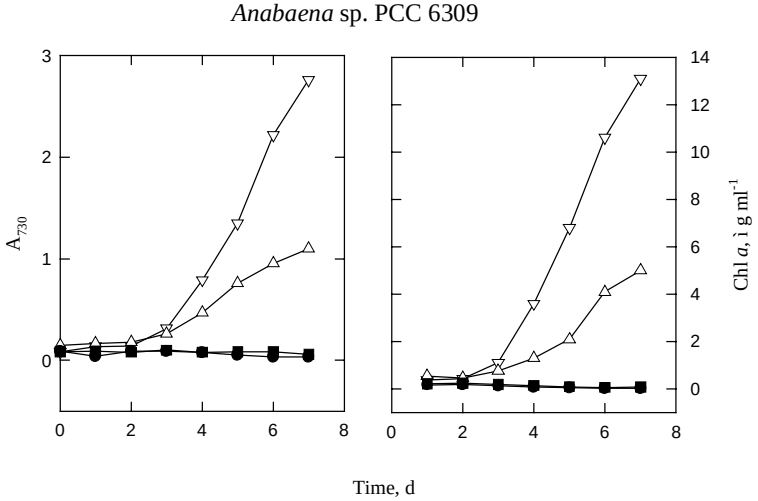

Fig. 2. Culture growth expressed in terms of turbidity A_{730} (A) and Chl a concentration (B) of N_2-fixing soil cyanobacterium *Anabaena* sp. PCC 6309 in the absence (▼) and in the presence of 0.2 mg (▲), 0.4 mg (■), 0.8 mg (●) epicuticular material/ml culture medium

These results indicated that the epicuticular material from *D. viscosa* decreased the activity of the anabolic process of photosynthesis, but they had no effect on photosynthetic electron transport.

Table 1. Effects of epicuticular material of *D. viscosa* leaves on the photosynthetic electron transport activities of N_2-fixing soil cyanobacterium *Nostoc sp.* PCC 7413. Photosynthetic activity was measured either as light-induced oxygen evolution in the presence of 1 mM phenyl-p-benzoquinone (PSII activity), or as oxygen uptake in the presence of 0.1 mM methyl-viologen (PSII plus PSI activity). Cell suspensions containing 4 μg Chl *a* ml^{-1}were used in the assays. Three independent measurements are averaged in each case (standard errors in parentheses).

	Relative photosynthetic activity ($H_2O \rightarrow CO_2$)	Relative photosynthetic electron transport ($H_2O \rightarrow MV$)	Relative PS II activity ($H_2O \rightarrow PBQ$)
Control	100	100	100
Epicuticular exudates (0.8 mg/ml)	36.3 (± 6.8)	95.3 (±15.3)	100 (± 4.2)

To analyse further the causes of the observed inhibitions in the cell proliferation, we investigated the effects of extracted epicuticulars from *D. viscosa* on the dark respiration of the N_2-fixing soil cyanobacteria. The respiratory activity was depressed to approx. 80% of the original rate (Table 2).

Finally we investigated the effects of *D. viscosa* epicuticulars on heterocyst formation by measuring the heterocyst per Chl *a* ratio in these cyanobacteria.

Table 2. Effects of epicuticular material of *D. viscosa* on the dark respiration of N_2-fixing soil cyanobacterium *Nostoc sp.* PCC 7413. Dark respiration was measured as oxygen uptake by cell suspensions (25 μg Chl *a* ml^{-1}). The cells were kept in darkness at 27 ^{0}C (about 18 min) until equilibrated before measuring O_2 uptake. Three independent measurements are averaged in each case (standard errors in parentheses).

	Relative dark respiration
Control	100
Epicuticular exudates (0.8 mg/ml)	77.5 (±2.5)

In the presence of 0.2 mg epicuticular material per ml culture, we observed an increase in the heterocyst Chla ratio, which was particularly dramatic in the case of Nostoc (Table 3).

Table 3. Effects of epicuticular material of *D. viscosa* on the heterocyst formation of N_2-fixing soil cyanobacteria. Each value represents the average with SE of results from three independent experiments.

	Relative heterocyst number of *Nostoc* sp. PCC 7413 (Number / ml culture μg Chl *a*)	Relative heterocyst number of *Anabaena* sp. PCC 6309 (Number / ml culture μg Chl *a*)
Control	100 (± 6.4)	100 (± 2.4)
Epicuticular exudates (0.2 mg/ml)	240 (± 12)	142 (± 4.9)

In a conclusion, leaf epicuticulars of *D. viscosa* are strong allelopathy agents for N_2-fixing soil cyanobacteria, decreasing dramatically the photosynthetic assimilation of CO_2 and increasing the heterocyst-to-vegetative cell ratio and most likely the assimilation of N_2.

Acknowledgements

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