South African Journal of Botany 92 (2014) 97–104

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Unique histochemical gradients in a photosynthesis-deficient plant gall

R.G.S. Carneiro [1], A.C. Castro [1], R.M.S. Isaias *

Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Botânica, Avenida Antônio Carlos, 6627, Pampulha, Belo Horizonte, Minas Gerais CEP: 31270-901, Brazil

ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form 20 December 2013
Accepted 24 February 2014
Available online 18 March 2014

Edited by JJM Meyer

Keywords:
Extralaminar galls
Functional designs
Metabolic gradients
Sucking-insect

ABSTRACT

Galls usually present low chlorophyll content, and their metabolism may vary depending on the *taxa* of the inducer and on the complexity of the gall structure. Primary and secondary plant metabolites allocated in gall tissues are evidenced with histochemical tests and may indicate the physiological status of such tissues. The histochemical and biochemical profiles of the galls induced by *Nothotrioza myrtoidis* Burck. (Hemiptera: Psylloidea) on *Psidium myrtoides* (Myrtaceae) were compared to those reported for some other Neotropical galls. The extralaminar galls of *N. myrtoidis* have low chlorophyll and nitrogen contents, but they accumulate more polysaccharides than the non-galled leaves. The histochemical gradient of reducing sugars is guaranteed by the activity of acid phosphatase, and ensures the nourishment of the gall inducer, while the gradients of phenolics and proanthocyanidins are both related to protection of the gall inducer and modulation of plant cell growth. The accumulation of reactive oxygen species seems to play a major role on the determination of the extent of tissue alterations during gall morphogenesis. The lack of morphological continuum and physiological continuum between the extralaminar galls of *N. myrtoidis* and the leaves of *P. myrtoides*, together with the low impacting feeding activity of the sucking insect, determine the establishment of a photosynthesis-deficient structure with unique features among Neotropical galls.

1. Introduction

The physiological traits of gall tissues have been evaluated through the quantification of photosynthesizing pigments and nutrients (Yang et al., 2003; Castro et al., 2012, 2013), and by histochemical tests (Hartley, 1998), providing an overview of the neo-established functionalities of plant tissues during gall morphogenesis. Different techniques have been used to evidence the metabolic dependence and the photosynthetic deficiency of galls (Yang et al., 2003; Khattab and Khattab, 2005). Even though the reduction on the concentration of chlorophylls is a widespread characteristic of galls, it has been recently demonstrated that photosynthesis is not strictly related to chlorophyll content, since normal rates of electron transport may be maintained, as occurred in the intralaminar galls on *Aspidosperma australe* (Oliveira et al., 2011a). On the other hand, Castro et al. (2012) demonstrated that the extralaminar horn-shaped gall on *Copaifera langsdorffii* has low chlorophyll content, but also acts as a sink of nutrients, as it does not photosynthesize at the same level of its host leaves. These findings indicate that morphology may interfere directly on gall metabolism.

The metabolic complexity of the insect-induced plant galls (Bronner, 1992) and the accumulation of plant metabolites related to the defense or nutrition (Hartley, 1998) were both assessed using histochemical tests. The study of Cecidomyiidae galls in the Neotropics, for instance, revealed conservative patterns on the accumulation of carbohydrate and related enzymatic activity (Oliveira et al., 2010, 2011b) both in intralaminar and extralaminar gall morphotypes. Galls induced by sucking-insects, on the other hand, present variable metabolic features. The cytological and histochemical gradients on the intralaminar galls of *Pseudophacopteron* sp. (Hemiptera) (Oliveira and Isaias, 2010) are similar to those of Cecidomyiidae galls, while the extralaminar bivalve-shaped galls induced by *Euphalerus ostreoides* (Hemiptera) on *Lonchocarpus muehlbergianus* (Fabaceae) present low carbohydrate metabolism (Isaias et al., 2011). As far as the accumulation of defensive compounds is concerned, the conspicuous structure of such extralaminar galls may be aposematic due to their high content of secondary plant metabolites, as previously proposed for galls on *Pistacia* (Inbar et al., 2010).

As seen, not only the insect *taxa* are important on the determination of gall metabolism, but also the gall structure, since the metabolism of the extralaminar galls tends to be lower than that of the intralaminar ones. At this scenario, the extralaminar galls induced by *Nothotrioza myrtoidis* Burck. (Hemiptera: Psylloidea), a recently described species from the Neotropics (Carneiro et al., 2013), on *Psidium myrtoides* (Myrtaceae) were studied to check the occurrence of such physiological

* Corresponding author. Tel.: +55 31 34092687; fax: +55 31 34092673.
E-mail addresses: rgscarneiro@gmail.com (R.G.S. Carneiro), arianechc@yahoo.com.br (A.C. Castro), rosy@icb.ufmg.br (R.M.S. Isaias).
[1] Tel.: +55 31 34092687; fax: +55 31 34092671.

http://dx.doi.org/10.1016/j.sajb.2014.02.011
0254-6299/

Table 1
Reagents used for histochemical detection of plant primary and secondary metabolites, reactive oxygen species and enzyme activity in hand-made cross sections of fresh non-galled leaves of *Psidium myrtoides* and galls of *Nothotrioza myrtoidis*.

Test/Reagent–Substance	Reaction mediums	Reference
Primary and secondary metabolites		
Fehling's reagent–reducing sugars	Equal parts of "A" (II copper sulfate 6.93% w:v) and "B" (sodium potassium tartrate 34.6% and 12% sodium hydroxide m:m:v) solutions heated to pre-boiling temperature	Sass (1951)
Lugol's reagent–starch	1% potassium iodine–iodide solution for 5 min	Johansen (1940)
Sudan red B–total lipids	Saturated solution of Sudan red B in 70°GL ethanol for 5 min	Brundett et al. (1991)
Coomassie blue–total proteins	0.25% Coomassie blue solution for 5 min	Dunn (1993)
Ferric chloride–phenolics	1% ferric chloride solution for 5 min	Johansen (1940)
DMACA–proanthocyanidins	Fixation: 0.5% caffeine sodium benzoate in 90% butanol for 1–2 h. Reaction: 1% *p*-dimethylaminocinnamaldehyde for up to 30 min.	Feucht et al. (1986)
NADI–terpenoids	1% α-naphthol, 1% dimethyl-*p*-phenylenediamine in 0.01 M phosphate buffer (pH 7.2) for up to 30 min	David and Carde (1964)
Jeffrey's mixture–alkaloids	Equal parts of 10% nitric acid and 10% chromic acid for 5 min	Johansen (1940)
Wiesner reagent–lignins	2% phloroglucinol in acidified solution for 5 min	Johansen (1940)
Reactive oxygen species		
Diaminobenzidine (DAB)–ROS	0.5% DAB solution for 15 to 60 min	Rossetti and Bonnatti (2001)
Enzyme activity		
Acidic phosphatase	Incubation: 0.012% lead nitrate and 0.1 M potassium sodium glycerophosphate in 0.5 M acetate buffer (pH 4.5) for 24 h, at room temperature. Reaction: Wash in distilled water and immerse in 1% ammonium sulfate for 5 min	Gomori (1956)
Glucose-6-phosphatase	Incubation: 20 mg of potassium glucose-6-phosphate in 125 ml of 0.2 M Tris–maleate buffer (pH 6.7), 3 ml of 2% lead nitrate in 7 ml of distilled water for 15 min to 2 h, at 37 °C. Reaction: wash in distilled water and immerse in 1% ammonium sulfate for 5 min	Jensen (1962)
Phosphorylase	Incubation: 1% glucose-1-phosphate in 0.1 M acetate buffer (pH 6.0) for 2 h at room temperature. Reaction: Lugol's reagent for 5 min	Jensen (1962)
Sucrose synthase	Fixation: 2% paraformaldehyde with 2% polyvinylpyrrolidone and 0.005 M dithiothreitol for 1 h. Incubation: 5 ml of 150 mM NADH, 5 ml (1 U) of phosphoglucomutase, 5 ml of 3 mM glucose-1,6-biphosphate, 5 ml (1 U) of glucose-6-phosphate dehydrogenase, 5 ml (1 U) of UDPG–pyrophosphorylase, 280 ml of 0.07% aqueous nitro-blue tetrazolium (NBT), 350 ml of buffer, and 50 ml of substrate for 30 min. Buffer: 100 mM HEPES, 10 mM $MgCl^2$, 2 mM EDTA, 0.2% BSA, and 2 mM EGTA at pH 7.4. Substrate: 0.75 M sucrose, 15 mM UDP, and 15 mM pyrophosphate.	Wittich and Vreugdenhil (1998)
Invertases	Incubation: 0.38 mM sodium phosphate (pH 7.5), 0.024% NBT, 0.014% phenazin metasulfate, 30 U of glucose oxidase, 30 mM of sucrose at room temperature for 3 h.	Zrenner et al. (1995) and Doehlert and Felker (1987)

and histochemical patterns already described for other galls. The following questions are addressed: (1) Do the biochemical and histochemical profiles of *N. myrtoidis–P. myrtoides* system fit the patterns described for other extralaminar galls? (2) Are there metabolic gradients in the extralaminar galls of the sucking-insect *N. myrtoidis*? (3) Do the histochemical profiles indicate the establishment of new functional designs in the tissues of the galls?

2. Materials and methods

2.1. Study area

The population of *P. myrtoides* O. Berg (Myrtaceae) infested by *N. myrtoidis* is located in a trail 2 km away from the park headquarters in the Reserva Particular do Patrimônio Natural Serra do Caraça,

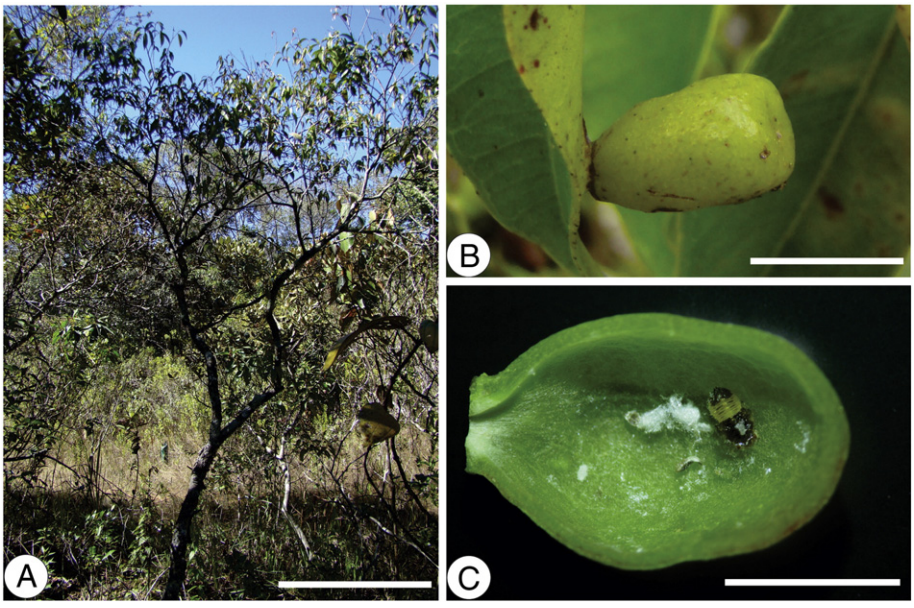

Fig. 1. Macroscopic characteristics of the *Psidium myrtoides–Nothotrioza myrtoidis* system. A—Habitus of *P. myrtoides*; B—Globoid leaf galls of *N. myrtoidis*; C—Gall in cross section evidencing the 4th instar nymph inside the broad nymphal chamber. Bars: A—70 cm; B and C—1 cm.