Thigmomorphogenesis in *Bryonia dioica*: Changes in soluble and wall peroxidases, phenylalanine ammonia-lyase activity, cellulose, lignin content and monomeric constituents*

G. DE JAEGHER¹, N. BOYER¹ and Th. GASPAR²

¹ Laboratoire de Phytomorphogenèse, Université de Clermont-Ferrand, 4, rue Ledru, F-63038 Clermont-Ferrand Cedex, France. ² Hormonologie fondamentale et appliquée, Institut de Botanique B22, Université de Liège – Sart Tilman, B-4000 Liège, Belgium

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Abstract. Rubbing young internodes of *Bryonia dioica* results in a reduced elongation and an increased diameter of the internodes. In the present study activities of some enzymes involved in the lignification process and levels of lignification were compared in rubbed and non-rubbed internodes. Rubbing caused an increase in the activities of phenylalanine ammonia-lyase and soluble and ionically- and covalently-bound cell wall peroxidases. Sensitivity of the covalently-bound wall peroxidase assay was markedly increased if syringaldazine was used as a substrate. Mechanical perturbation induced an increase in lignin, lignin monomer (sinapylic, coniferylic and p-coumarylic alcohols) content and the number of lignifying vessels. Conversely, rubbing resulted in a decrease in cellulose content. The hypothetical interpretation of the thigmomorphogenetic response through cell wall lignification and hence rigidification is consistent with all the presented results. A comparison is possible between this accelerated lignification and induced lignification as a mechanism of disease resistance. The thigmomorphogenetic response in *Bryonia dioica* can be considered as a mechanism of resistance in order to withstand further environmental mechanical perturbation.

Introduction

The most common thigmomorphogenetic response, which is found in a wide variety of plant species, is a retardation of elongation accompanied by an increase in thickness [17]. Mechanical stress exerted on the limbs and trunks of conifers and woody dicotyledons results in a unique developmental response resulting in the production of reaction wood [5, 27]. In young rubbed *Bryonia dioica* internodes, inhibition of growth has been shown to be correlated with both a rapid rise in activity of the basic peroxidases, which in turn could have induced a decrease in IAA [16], and a more progressive increase in activity of the acidic peroxidases [6]. The thigmomorphogenetic response has been demonstrated to be mediated by ethylene [3, 7, 27].

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Address for correspondence: Geert De Jaegher, Laboratoire de Phytomorphogenèse 4, rue Ledru, F-63038 CLERMONT-FERRAND Cedex France.

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Treatment of plants with exogenously applied ethylene or with the ethylene-releasing agent ethephon mimics the effect of mechanical perturbation [3, 7] and induces also an increase in peroxidase activity [7].

Since the changes in peroxidase activity in rubbed internodes were similar to those occurring naturally in non-rubbed Bryonia internodes increasing in age, thigmomorphogenesis appeared to operate as an accelerated ageing process. Thus, a working hypothesis can be formulated relating inhibition of growth to cell wall rigidification through lignification. Much evidence links ethylene to tracheary element differentiation and lignification [26], so that in the thigmomorphogenetic response an increased ethylene production might induce modifications in the developing secondary xylem.

The aim of this work was to compare the levels of lignification and activities of some enzymes involved in the lignification process in rubbed and non-rubbed internodes and to discuss the relevance of any differences to growth retardation caused by mechanical stress and eventually mediated by ethylene.

Wall peroxidase activity and number of lignifying vessels were both investigated through parallel biochemical and histochemical techniques using syringaldazine as a specific staining substrate for peroxidases involved in the lignification process [11, 14].

Materials and methods

Plant material and growing conditions

Young plants of Bryonia dioica were raised from seeds in a controlled environment room (16 h light daily at 18 W.m⁻², 25 °C, 70% humidity) in vermiculite. When the plants had developed two internodes, they were transferred to a mineral solution, at least 48 h before the rubbing treatment. The first internode, which was approximately 12 mm long, was held at the top and gently rubbed between the thumb and forefinger for 3 sec.

Peroxidase fractionation, activity and electrophoretic separation

Enzymatic extractions were performed as described previously [6] with some modifications. Fresh tissue was ground in 30 mM phosphate buffer at pH 7 with a buffer-to-tissue ration of 0.5 ml:1 g fresh weight. This ratio was maintained during the whole extraction procedure. The homogenates were centrifuged at 3000 g for 10 min and the resulting supernatants were assayed for soluble peroxidase activity. The pellets were washed with extraction buffer and centrifuged in the same way. The resulting supernatants were discarded. To release ionically-bound cell wall enzymes, the 3000 g pellets were dispersed in extraction buffer containing 1 M NaCl followed by centrifuging at 3000 g for 10 min. The resulting supernatants were used as the ionic