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A Study of Some Biochemical and Histopathological Responses of Wet-stored Recalcitrant Seeds of *Avicennia marina* Infected by *Fusarium moniliforme*

VESSELINA S. ANGUELOVA-MERHAR, CLAUDIA CALISTRU and PATRICIA BERJAK*
School of Life and Environmental Sciences, University of Natal, Durban, 4041 South Africa

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Although fungi cause a recognized problem during storage of recalcitrant seeds of many tropical species, there are no data to date on defence strategies of these seeds against fungal attack. To ascertain whether recalcitrant seeds of *Avicennia marina* elaborate compounds that might suppress fungal proliferation during hydrated storage, the production and efficacy of β-1,3-glucanase (EC 3.2.1.39) and chitinase (EC 3.2.1.14) were studied in relation to histopathological changes. Freshly harvested seeds had low β-1,3-glucanase and chitinase activities and fluorescence microscopy revealed progressive deterioration of the internal tissues of these seeds associated with fungal infection during hydrated storage. In seeds treated to minimize associated fungi (clean seeds), β-1,3-glucanase and chitinase activities increased significantly during 10 d of hydrated storage. Similar high levels of activity were observed when these seeds were experimentally infected with *Fusarium moniliforme* and subjected to further storage. The histopathological observations indicated delayed disease development in the 10-d clean-storage period, although the hypersensitive response was not observed. The results suggest that, although the recalcitrant seeds of *A. marina* elaborate some antifungal enzymes, there is a lack of effective defence strategies that might lead to successful responses against fungal infections.

Key words: *Avicennia marina*, chitinase, fluorescence microscopy, *Fusarium moniliforme*, β-1,3-glucanase, hydrated storage, recalcitrant seeds.

INTRODUCTION

A number of economically important tropical and subtropical crops, e.g. tea (Berjak *et al.*, 1993), mango, litchi and commercial rubber (Chin and Roberts, 1980), as well as forest and horticultural species, are characterized by producing recalcitrant seeds. Recalcitrant seeds are not only hydrated when they are shed, but are continuously metabolically active. As such, most are akin to seedlings, and bear little resemblance to the familiar quiescent, desiccated orthodox seed (Berjak *et al.*, 1989; Berjak and Pammenter, 2001). Recalcitrant seeds are highly sensitive to desiccation and some are also chilling sensitive, necessitating storage under high relative humidity conditions and at ambient temperatures. This approach, termed wet or hydrated storage is, however, conducive to fungal proliferation that severely curtails the post-harvest lifespan of seeds (Calistru *et al.*, 2000). Although the role of fungi in deterioration during storage of recalcitrant seeds has been the focus of several reports (Mycock and Berjak, 1990, 1995; Pongapanich, 1990; Singh and Singh, 1990; Berjak, 1996; Calistru *et al.*, 2000; Sutherland *et al.*, 2002), there are no published data about the defence strategies of such seeds against fungal attack.It is well known that the plants react to microbial infection with a broad range of defence mechanisms, which may restrict or prevent pathogen growth. Among these are cell wall reinforcement (Hahn *et al.*, 1989), accumulation of antimicrobial proteins (Fritig *et al.*, 1998), production of phytoalexins (Ebel, 1986) and the hypersensitive response (Hammond-Kosak and Jones, 1997), which are all important strategies contributing towards a successful resistance response. Genetic deficiencies in the ability of the plant to activate these various pathways are commonly associated with enhanced susceptibility.The present contribution is a part of an ongoing study on the mechanisms associated with deterioration, and the nature of the responses of recalcitrant seeds to experimental inoculation with *Fusarium moniliforme* (the most deleterious of the local recalcitrant-seed-associated fungal species). The highly recalcitrant seeds of *Avicennia marina* (Forssk.) Vierh. lose viability within 14–16 d in wet storage (Berjak *et al.*, 1989) and within 7 d if inoculated with *F. moniliforme* immediately after harvest. However, if wet-stored for 4 d before inoculation, the seeds become less susceptible to the depredation associated with the presence of this fungus (Calistru *et al.*, 2000), but the effect is transient. The present study was undertaken to assess histopathologically the location and extent of the infection caused by *F. moniliforme* in *A. marina* seeds at different stages during hydrated storage and also to ascertain if, and when, these recalcitrant seeds elaborate compounds that might suppress fungal proliferation during storage. The role of two pathogenesis-related (PR) proteins [β-1,3-glucanase (EC 3.2.1.39) and chitinase (EC 3.2.1.14)] was evaluated and related to the short-term storage responses of these seeds. The antifungal properties of β-1,3-glucanases and chitinases have been discussed extensively (for reviews, see Bol and Linthorst, 1990; Stintzi *et al.*, 1993; Van Loon,

* Correspondence. Fax +27 (0) 31 260 1195, e-mail berjak@biology.und.ac.za