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Short communication

An ultrastructural investigation of the surface microbiota present on the leaves and reproductive structures of the resurrection plant *Myrothamnus flabellifolia*

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Abstract

The leaves, flower and stems of the southern African angiosperm resurrection plant *Myrothamnus flabellifolia* were investigated at the ultrastructural level to determine the source of previously reported fungal contamination. Fungal mycelia and hyphae of the genera *Aspergillus* and *Penicillium* were found localized to the hydathodes of the leaves and stigmatic surfaces of the female flowers in both desiccated and hydrated specimens. A waxy bacterium of the genus *Bacillus* was found to colonise the waxy epidermal surfaces of the leaves and flowers which was also where fungal cells were found to be absent. It is suggested that the wax like deposits within the leaves and stems as well as over the epidermal surface prevent the growth of the fungal organisms. These fungi opportunistically invade moist surfaces, such as the floral stigmas, during periods of moisture availability and may thus negatively impact plant development.

Keywords: Aspergillus; Bacillus; Desiccation tolerance; Hydathodes; Penicillium; Wax layers

1. Introduction

Resurrection plants are a unique group of plants found predominantly in the arid and semi-arid areas of the world and so southern Africa possesses a rich diversity of these plants (Gaff, 1971). A remarkable feature of these plants is their ability to survive extensive dehydration (desiccation) of their vegetative organs (e.g. leaves) without suffering permanent injury (Gaff, 1971; Farrant, 2000). *Myrothamnus flabellifolia* Welw. is considered to be the largest resurrection plant and is unique as being the only member possessing a woody stem (Moore et al., 2007a). It has a widespread distribution across southern and eastern Africa where it is located on rock inselbergs composed of granite, shale and quartz (Moore et al., 2005, 2007a). The plant possesses fan-like leaves which fold along the stem exposing the pigmented and waxy abaxial surface to the environment (Moore et al., 2007b). These fan-like leaves unfold and change colour from dull-brown to green during the summer rainy season when the plants absorb water run-off from the rocky slopes via their roots (Moore et al., 2007b). The plants exist singly or in colonies and are dioecious (Child, 1960). Numerous attempts have been made to cultivate transported plants in greenhouse conditions in order to study the mechanisms of desiccation tolerance in more detail (Goldsworthy, 1992; Glen et al., 1999). However, it was discovered that plants transported out of their native habitats were susceptible to many problems such as disease (e.g. red spider mite infection), seasonal variation effects, soil composition, temperature effects and sub-optimal light intensities (personal observations, J.P. Moore and K. Cooper). There have, however, been successes with cultivating *M. flabellifolia* in a humid atmosphere which appeared to improve survival conditions (Glen et al., 1999). A common observation of rehydrating twigs from *M. flabellifolia* is that prolonged presence in a humid atmosphere promotes extensive fungal growth over the leaves (Child, 1960; personal observation, J.P. Moore). This was previously reported by Child

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(1960) who stated that no obvious source of fungal infection could be found even after a detailed examination of the leaves and branches. To clarify the source and identity of this fungal contamination we undertook an ultrastructural study of the leaves, stem and reproductive structures of *M. flabellifolia* with the further aim of ascertaining the role these microbes may play during plant revival and growth. This is of particular importance considering that *M. flabellifolia* is endangered due to excessive collection for traditional medicine formulations (Moore et al., 2007a; Van Wyk et al., 1997).

2. Materials and methods

2.1. Plant material

M. flabellifolia plants, collected from the Buffelskloof Nature Reserve, Mpumalanga Province, South Africa, and Outjo, Namibia were maintained in a glasshouse in the Botany Department, University of Cape Town. Excised twigs/leaves from plants kept in a separate section of the glasshouse, were either incubated for 24–48 h in a humid atmosphere at room temperature to induce fungal growth or impregnated on LB agar plates and incubated at 25 and 37 °C for 24–48 h.

2.2. Light and scanning electron microscopy

The material for ultrastructural analysis was prepared as previously described (Moore et al., 2006, 2007b). Photographs were taken with a Wild Photomakroscop equipped with an AxioCam digital camera. Scanning electron microscopy was performed using a Leica Stereoscan 440 digital scanning electron microscope equipped with a Fisons LT7400 Cryo transfer system. Certain specimen-containing stubs were coated with palladium–gold prior to viewing.

3. Results

The unusual fan-like appearance of *M. flabellifolia* leaves has been reviewed and discussed previously (Moore et al., 2007a). The concertina-like structure of the leaves, supported by sclerenchymous ribs, is believed to permit controlled folding and tissue compaction upon rehydration whilst a rapid return to the full turgor state at rehydration (Moore et al., 2007b). This can be seen in hydrated (Fig. 1A) and desiccated (Fig. 1E) leaves. Similarly the reproductive structures have been discussed previously with respect to understanding the pollination and seed dispersal mechanisms of the plant in relation to its unique desiccation-associated ecology (Moore et al., 2007a). The male flowers are arranged in catkin-like inflorescences which bear anthers on short lateral filaments (Fig. 1A, B). The purplish-red anthers dehisce longitudinally releasing large amounts of yellow pollen grains (Fig. 1B). This pollen is believed to be carried to the female flowers possibly via a combination of both insect and wind driven mechanisms (Moore et al., 2007a). The female flowers are composed of three carpels with either pink (Fig. 1C) or red-purple (Moore et al., 2007a) stigmatic surfaces which possess a feathery appearance (Fig. 1D). Floral maturation, pollination and seed dispersal are naturally believed to occur during the summer rainy season when the plants are fully hydrated. Desiccated flowers present on the branches of desiccated plants are found in a state of post-anthesis for male plants and post-pollination or post-seed-dispersal for female ones. Desiccated female flowers appear dark brown, shriveled and the stigmatic surfaces are devoid of colour as well as the previously observed feathery appearance (Fig. 1E, F). Similarly, male florescences are light brown with dried out anthers and little to no pollen present (Fig. 1G). Desiccated fruits are occasionally also found composed of brown bulbous carpels containing numerous minute ‘dust-like’ seeds (Fig. 1H). Visual inspection as well as scrutiny of the material under the dissecting microscope revealed no obvious source of the fungal contamination observed during prolonged exposure to high humidity. Further analysis under a higher magnification using scanning electron microscopy was therefore performed.

Detailed ultrastructural analysis of both desiccated and hydrated leaves of *M. flabellifolia* has been performed (see Moore et al., 2006, 2007b). An interesting feature of *M. flabellifolia* leaves, apart from the fan-like corrugations at the leaf surface and sclerenchymous ribs, is the presence of hydathode-like structures (which appear to be formed from modified stomata) at leaf apices (Fig. 2A; Moore et al., 2007b). The presence of desiccated salt-like deposits at the apex of desiccated leaves (Fig. 2B) provides strong support to the suggestion that these hydathode structures may function to regulate the salt and solute content of the leaves particularly during desiccation and rehydration (Moore et al., 2007b). Higher magnification examination under electron microscopy of the apices of desiccated leaves revealed clear evidence of microbial presence (Fig. 2C). The desiccated droplet of salts present near the hydathodes of desiccated leaves appeared to be covered with rod shaped microbes and fungal hyphae (Fig. 2C). Furthermore, the fungal hyphae were shown to emerge from fold crevices present at the desiccated leaf surface (Fig. 2D) suggesting that the fungi are protected within the folds of the plants leaves. Incubation of leaf segments either in a humid atmosphere or on nutrient agar Petri plates (see Material and Methods) confirmed the emergence of significant fungal growth from the apices and to a lesser extent the leaf folds (Fig. 2E). The fungal mycelium appeared to rapidly spread across the leaf surface producing aerial fruiting bodies (Fig. 2E). To ascertain if the fungus was parasitic or saprophytic on the plant, closer examination of the fungal infected material was undertaken (Fig. 2F). No evidence of penetration of the fungal hyphae into the leaf surface was observed. Copious fruiting bodies emerged from the fungal hyphae growing on the leaf surface (Fig. 2G) and these in turn were composed of numerous fungal spores. Higher magnification of the fruiting bodies revealed spores which bore ‘wheel’ shaped structures that possessed clear ridges spanning their circumference at set intervals (Fig. 2H). The fungal organisms present on the leaf surface were identified as those belonging to the genus *Aspergillus* and *Penicillium* (personal communication, S. Coertze, Department of Plant Pathology, Stellenbosch University). Confirmation of fungal presence on the leaves of *M. flabellifolia* suggested that further analysis be undertaken on the reproductive organs to ascertain if fungi were associated with these structures also.

Scanning electron microscopy of hydrated female flowers revealed similar surface morphology to that observed previously