Evidence for the role of phytophagous insects in dispersal of non-grass fungal endophytes

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Fungal endophytes were isolated from the leaves of *Calotropis gigantea* (milkweed). *Colletotrichum gloeosporioides* was most frequently isolated over the 24 month study period as an endophyte and it contributed to about 45% of the total number of endophyte isolates obtained. *Poecilocerus pictus* (painted grasshopper) which feeds on the leaves of milkweed neither avoided nor preferred milkweed leaves coated with a spore suspension of *C. gloeosporioides*. Three of the endophytes tested passed through the gut of the insect without being digested and retained their viability. This suggests that phytophagous insects could serve as an agent for the dispersal of non-grass fungal endophytes in plant communities such as tropical forests.

Key words: fungal endophytes, endophyte transmission, phytophagous insects

Introduction

Non-clavicipitaceous endophytes cause discrete and symptomless infections in the aerial tissues of plants and survive within these tissues at least for part of their life cycle. Unlike the clavicipitaceous endophytes that are restricted to grasses and are often transmitted vertically, the non-clavicipitaceous endophytes infect a broad range of plant hosts belonging to disparate groups and families and are transmitted horizontally. These endophytes are taxonomically restricted to the ascomycetes and their anamorphs and span several ecological functional groups such as mutualists (Redman *et al*., 2002; Arnold *et al*., 2003), commensals (Deckert *et al*., 2001) and latent pathogens (Sinclair and Cerkauskas, 1996; Photita *et al*., 2004, 2005; Gonthier *et al*., 2006; Suryanarayanan and Murali, 2006).

Recent studies have shown that the leaves of tropical plants are densely colonized by endophytes although their species richness varies with the type of the tropical plant communities (Arnold *et al*., 2003; Suryanarayanan *et al*.,

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2003; Kumar and Hyde, 2004; Promputtha *et al*., 2005; Wang *et al*., 2005). Compared to the data that have accumulated on the biology of clavicipitaceous endophytes, the information that we have on non-clavicipitaceous endophytes is less. There are also very few experimental studies on the role phytophagous insects in the dissemination of such endophytes. Here, we discuss the possible role of the Painted grasshopper (*Poecilocerus pictus*) in passively aiding the dispersal of foliar fungal endophytes of milkweed (*Calotropis gigantea*). This plant belongs to the family *Asclepiadaceae*; it is a large shrub found throughout India in dry, waste grounds. All parts of this plant are used as medicine in the indigenous system of medicine, the Ayurveda (Warrier *et al*., 1994). *Poecilocerus pictus* is one of the many insects associated with *Calotropis gigantea*. This grasshopper feeds on the leaves of milkweed and, at times, entirely defoliates the plant.

Materials and methods

Sampling of leaves for endophytes

Mature, healthy leaves of *Calotropis gigantea* plants growing in the city of Chennai were screened for endophyte presence on a monthly basis for 24 consecutive months (January 2001-December 2002). Every month, 40 leaves were collected, washed thoroughly in running tap water, and from each leaf, three segments (0.5 cm^2) were cut from the apical, middle and basal portions of the midrib and screened.

Surface sterilization and isolation of endophytes

The 120 leaf segments obtained each month were surface sterilized by dipping them in 70% ethanol for 5 seconds, followed by treatment in sodium hypochlorite (4% available chlorine) for 60 seconds and finally rinsed in sterile water for 10 seconds (Dobranic *et al*., 1995). Of these, 100 surface sterilized segments were plated on Potato Dextrose Agar medium contained in Petri dishes. Ten segments were placed equidistantly in each Petri dish containing 20 ml of the medium amended with chloramphenicol (150 mg/L^{-1}). The Petri dishes were sealed with Parafilm^{TM} and incubated in a light chamber for 21 days at 26ºC (Bills and Polishook, 1992). The light regime provided was 12 hours light: 12 hours darkness from cool white, daylight fluorescent lamps (Suryanarayanan *et al*., 1998). The colonization frequency (CF%) was calculated following the method of Hata and Futai (1995).