

COLONIZATION OF *BOTRYODIPLODIA THEOBROMAE* PAT. IN RUBBER SEEDS

Seeds of the rubber tree, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg., are infected by a large number of fungal pathogens (Srivastava, 1956; Urben *et al.*, 1982; Singh and Singh, 1987). *Botryodiplodia* is a common pathogen of rubber causing die-back and collar-rot. Gorenz (1953) reported *B. theobromae* on maturing seed-pods in Mexico. Srivastava (1956) intercepted it in seeds from Malaysia. Urben *et al.* (1982) reported *B. theobromae* and six other fungi in rubber seeds from Brazil. Histopathology of *B. theobromae* infected rubber seeds and host parasite relationship are unknown and hence the present study was undertaken.

Rubber seeds of two cultivars, RRIM 607 and RRIM 600 from Panjam Estate, Negri Sembilan, were used. Seeds, pretreated with one per cent mercuric chloride for 15 min were decoated, split longitudinally and one half of each seed was plated (one seed/plate) using standard blotter technique (Anon., 1976). The other halves were fixed in formalin acetic alcohol. Counterparts of seed halves which yielded pure growth of *B. theobromae* were used for histopathological studies. Established methods of clearing of seed tissues and microtome sectioning were used (Singh *et al.*, 1977; Singh, 1983). Safranin-light green combination was followed for staining (Johanson, 1940).

The rubber seed is composed of a woody testa (separated in decoated seeds), massive endosperm (Fig. 2) and dicotyledonous embryo. The infected seeds had brown black discoloration progressing from micro-

pylar to chalazal end. The cleared whole-mount preparations of moderately infected seeds showed the occurrence of thick, brown, septate and knotty mycelium in tegmen, perisperm, endosperm and embryo while those of black discoloured kernels contained abundant mycelium all over (Fig. 1).

The microtome sections revealed the quantitative differences in weakly, moderately and heavily infected seeds with respect to the extent of invasion of mycelium in different seed components. The fungus colonized tegmen, perisperm, endosperm and embryo in all the symptomatic seeds. But in the asymptomatic seeds its presence was recorded only in sections of one out of eight kernels examined. Light coloured, scanty mycelium occurred in the sections of such seeds in tegmen and perisperm only. Among the discoloured kernels, thick-walled, septate, branched, brown hyphae were recorded in tegmen, perisperm and endosperm in kernels with mild infection. The mycelium was thin and hyaline in embryo. No mycelium was recorded in vascular bundles and latex cells in tegmen and cotyledons. In moderately and heavily infected seeds abundant mycelium occurred in various tissues. In the former, a few outer layers of tegmen were compressed and distorted and the mycelium was inter- as well as intracellular. The cells in the remaining layers of tegmen and those of perisperm showed signs of loosening and depletion of cytoplasm (Fig. 3). Hyphae occurred in tracheids of vascular bundles in tegmen (Fig. 4). The fungus formed a