A Review of *Lasiodiplodia theobromae* with Particular Reference to its Occurrence on Coniferous Seeds

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**SYNOPSIS**

The fungal pathogen *Lasiodiplodia theobromae* has been associated with numerous plant diseases on a wide variety of hosts. Conifers seem to be particularly susceptible to infection, although the true parasitic status of the fungus is still largely unresolved. In South Africa, *L. theobromae* is associated with discolouration and reduced germination of *Pinus elliottii* seeds. The mode of seed infection is, however, still unclear. This review attempts to critically summarise current knowledge pertaining to infection of coniferous species by *L. theobromae*, with special emphasis on its ability to act as a seedborne pathogen and means of controlling it.

**INTRODUCTION**

The fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. (synonyms: *Botryodiplodia theobromae* Pat., *Diploidi a natalensis* Pole Evans.) is pleomorphic, plurivorous and ubiquitous in the tropics and subtropics (Punithalingam 1979). For this reason, it is referred to in the literature by a multiplicity of names (Punithalingam, 1976, 1979). Although it has on a few occasions been referred to as the pycnidial state of *Physalospora rhodina* Cooke, the ascomycete state of *L. theobromae* is relatively uncommon on living plants and plays no part in infection (Punithalingam, 1979). It is therefore appropriate to refer to the fungus only by the conidial state.

*L. theobromae* has been implicated as the primary cause of many plant diseases. Its true parasitic status is, however, large unresolved and it is predominantly regarded as a facultative wound pathogen. A critical assessment of reports on plant diseases attributed to *L. theobromae* was made by Punithalingam (1979).

The notoriety of *L. theobromae* as a seedborne pathogen is well documented and it appears that coniferous species are particularly susceptible to infection (Table 2). The association of *L. theobromae* with discolouration of coniferous seeds (Carneiro, 1986; Rees, 1988; Fraedrich and Miller, 1989), is currently of particular importance to South African forestry. Significant problems have been experienced in this country with reduced germination of discoloured seeds of *Pinus elliottii* from which *L. theobromae* has been isolated (Figures 1 and 2). The aim of this paper is to review the occurrence of *L. theobromae* as a pathogen of forest tree species, especially coniferous species. Special emphasis will be given to its occurrence as a seedborne pathogen of *Pinus* spp. This will serve as a basis for future research on this problem in South Africa.

**BIOLOGY**

**Morphology and physiology**

*Lasiodiplodia theobromae* is a member of the Coelomycetes having pycnidia that are immersed to erumpent or superficial, pilose or glabrous, simple or often aggregated reaching up to 5 mm wide, with or without a stroma (Figure 3) (Punithalingam, 1979). Colonies of the fungus on oat-meal agar are greyish and fluffy with abundant aerial mycelium; the reverse of the colony ranges from fuscous to black (Figure 4) (Punithalingam, 1976). Conidiogenous cells are holoblastic, annelidic and the conidia are initially hyaline, unicellular, ellipsoidal to oblong, thick-walled with granular contents (Punithalingam, 1979). Mature conidia are two-celled, cinnamon to fawn or dark brown, usually 20 to 30 by 10 to 15 micrometers in size, with differentially pigmented longitudinal bands resembling striations (Figure 5) (Punithalingam, 1979). Numerous studies have been conducted on the various physiological aspects of *L. theobromae* including effects of light, pH, temperature, carbon and nitrogen sources and vitamin requirements (Punithalingam, 1979; Webster et al., 1971; Rao and Singhal, 1978). Optimum conditions for the growth of mycelium and formation of pycnidia are photoperiods of up to 16 hours daily; less than four hours light daily over a period of 23 days inhibits sporulation (Perera and Lago, 1986). Conidia are released from pycnidia...
following either wetting or drying and are single-celled at time of discharge whereafter they become two-celled. *L. theobromae* produces extracellular cellulolytic and pectic enzymes and an antibiotic, botryodiplodin (Punithalingam, 1979).

**Symptomology and pathology**

*Lasiodiplodia theobromae* causes a wide variety of symptoms on numerous coniferous and other tree species of all ages. Tip dieback of *Pinus taeda* L. and *P. elliottii* Englm. seedlings that turn purple and eventually die has been attributed to the pathogen (Rowan, 1982). Infection of mature trees by *L. theobromae* is mostly due to mechanical damage often caused by insects (Agarwal and Sinclair, 1987). *P. elliottii*, *P. taeda* and *P. caribaea* P.M. Morelet trees afflicted with root rot revealed the presence of *L. theobromae* in the roots although *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton was confirmed to be the primary pathogen (Zhong and Liang, 1990). *L. theobromae* has also been found to cause blue stain of *P. massoniana* Lamb. and *Hevea brasiliensis* Mull.Arg. (Fuetal., 1988). Symptoms of needle cast of *P. elliottii* caused by *L. theobromae* have been observed; the infected needles displaying spots or lesions (Shayesta and Rahman, 1985). *Eucalyptus* seedlings infected with *L. theobromae* show symptoms of physiological wilting such as drooping apical shoots with flaccid leaves (Sharma et al., 1985).

The association of fungi with seeds is well documented and *L. theobromae* has often been implicated in this regard having been found to be borne both internally and externally on seeds of numerous forest tree species (Table 2). Dayan (1986) isolated 10 fungal genera comprising 15 species from 31 species of forest tree seeds. Coniferous species appear to be particularly susceptible with a wide range of fungi having been identified. Mittal and Wang (1987) isolated 13 species of fungi from eastern white pine seeds and 17 species from white spruce. The needle pathogen *Sirococcus strobilinus* was found in seeds of *Picea* sp. (Wicklow-Howard and Skujins, 1980; Sutherland et al., 1981). *Diplodia* sp. and *Fusarium*
TABLE 1. The occurrence of *L. theobromae* as a forest tree pathogen

<table>
<thead>
<tr>
<th>Host</th>
<th>Symptoms and signs</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hevea brasiliensis</em> Mull. Arg.</td>
<td>Blue-strained timber, dieback</td>
<td>Fu et al. (1988)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Narain and Dash (1989)</td>
</tr>
<tr>
<td><em>Pyrus</em> spp.</td>
<td>Canker and dieback</td>
<td>Avtar et al. (1990)</td>
</tr>
<tr>
<td><em>Cornus</em> spp.</td>
<td>Cankers carrying conidia</td>
<td>Mullen et al. (1991)</td>
</tr>
<tr>
<td><em>Platanus occidentalis</em> L.</td>
<td>Production of cankers</td>
<td>Filer (1969)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lewis et al. (1978)</td>
</tr>
<tr>
<td><em>Elaeagnus angustifolia</em> L.</td>
<td>Bark, cambium and phloem tissue killed</td>
<td>Peterson (1976)</td>
</tr>
<tr>
<td><em>Aleurites montana</em> Lour.</td>
<td>Canker disease</td>
<td>Large (1948)</td>
</tr>
<tr>
<td><em>Eucalyptus</em> spp.</td>
<td>Root collar canker causing physiological</td>
<td>Sharma et al. (1985)</td>
</tr>
<tr>
<td></td>
<td>wilting</td>
<td>Soni et al. (1991)</td>
</tr>
<tr>
<td><em>Citrus reticulata.</em> Blanco.</td>
<td>Twig gumming and dieback</td>
<td>Feder and Huchins (1966)</td>
</tr>
<tr>
<td><em>Carica papaya.</em> L.</td>
<td>Fruit rot</td>
<td>Hunter et al. (1969)</td>
</tr>
<tr>
<td><em>Citrus aurantifolia.</em> Swingle.</td>
<td>Gummosis and dieback</td>
<td>Singh et al. (1971)</td>
</tr>
<tr>
<td><em>C. sinensis</em> Osbeck</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Carica papaya.</em> L.</td>
<td>Stem-end rot</td>
<td>Hunter et al. (1969)</td>
</tr>
</tbody>
</table>

Moniliforme Sheldon have been isolated from Japanese black pine (*Pinus thunbergii* Parl.) and red pine (*P. resinosa* Aiton.) seeds and found to be pathogenic (Watanabe, 1988). Seeds of *P. elliottii*, *P. thunbergii* and *P. resinosa* were found to carry *Fusarium moniliforme* and *Diplodia* sp. internally (Anderson et al., 1984; Watanabe, 1988). Seeds of *P. elliottii* and *P. taeda* have been reported to harbour a larger variety of fungi compared to the seeds of other *Pinus* spp. (Carneiro, 1986).

Coniferous seeds infected by fungal pathogens display a wide range of symptoms ranging from malformation, discolouration of the testa and internal decay (Neergaard, 1977). Germination failure inevitably results from infection as in the case of *Ceniculodendron pyriforme* Salt. isolated from seeds of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm) showing varying amounts of discolouration of the seed surface (Wicklow-Howard and Skujins, 1980). Over one-third of the embryos from seeds of *P. caribaea* with infected endosperms were found to be decayed and fragments of hyphae found in the cavity between the endosperm and embryo were identified as *L. theobromae* (Reeds, 1988). Inspection of the seed surface using SEM revealed that 25% of the seeds were externally contaminated by conidia of *L. theobromae*. Similar observations were made for seeds of *P. elliottii* infected with *L. theobromae* where the infected seeds had a black discolouration and germination percentages were reduced (Fraedrich and Miller, 1989).

**MODE OF SEED INFECTION**

Seeds are generally infected via the seed primordium, either directly from the mother plant, or by transmission from the outside (Neergaard, 1977). According to Agarwal and Sinclair (1987) seeds may also be infected by penetration of fungal pathogens through the intact ovary wall and seed coat or through natural openings or injuries. The location of *L. theobromae* inside *P. caribaea* seeds led Rees (1988) to suspect that infection occurred during pollination when the ovule was fertilised by an internally infected pollen grain.

Harvesting and storage may play an important role in the infection of seeds by fungi (Agarwal and Sinclair, 1987). Christensen (1973) noted that numerous storage fungi could cause seed discolouration and weakening or mortality of embryos. Conditions that promote the growth of fungi on seeds in storage are high moisture and temperature, the amount of debris and foreign material present in seedlots, and
TABLE 2. The occurrence of *L. theobromae* as a seedborne pathogen

<table>
<thead>
<tr>
<th>Host</th>
<th>Disease or symptom</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus taeda</em> L.</td>
<td></td>
<td>Carneiro (1986)</td>
</tr>
<tr>
<td><em>Pinus elliottii</em> Englm.</td>
<td></td>
<td></td>
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<tr>
<td><em>Pinus caribaea</em> P.M. Morelet.</td>
<td></td>
<td>Rees (1988)</td>
</tr>
<tr>
<td><em>Pinus elliottii</em> Englm.</td>
<td>Seed embryo's are decayed</td>
<td>Fraedrich and Miller (1989)</td>
</tr>
<tr>
<td><em>Pinus thunbergii</em> Parl.</td>
<td>Black seeds rot</td>
<td>Watanabe (1988)</td>
</tr>
<tr>
<td><em>Pinus resinosa</em> Aiton.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dolichos biflorus</em></td>
<td>Seed rot and seedling</td>
<td>Maholay and Sohi (1977)</td>
</tr>
<tr>
<td><em>Glycine max.</em> Merr.</td>
<td>Reduced seed germination</td>
<td>Ellis <em>et al.</em> (1977)</td>
</tr>
<tr>
<td><em>Lagenaria siceraria</em> Standl. and <em>Cucurbita</em> spp.</td>
<td>Dry black rot of squash and blackening of seeds</td>
<td>Maholay and Sohi (1982)</td>
</tr>
<tr>
<td><em>Hevea brasiliensis</em> Mull. Arg.</td>
<td>Pink dorsal surface of seeds</td>
<td>Srivastava (1964)</td>
</tr>
<tr>
<td><em>Musa balbisiana</em> L.</td>
<td>Seed rot</td>
<td>Goos <em>et al.</em> (1961)</td>
</tr>
</tbody>
</table>

the presence and activity of insects or mites (Christensen, 1973). Latent infection of newly harvested seeds will thus also be influenced by storage conditions. Visible symptoms such as discoloration may therefore only appear after some time has elapsed under adverse storage conditions. Studies to determine when and how seeds or cones of *P. elliottii* become infected by *L. theobromae* are therefore urgently required to determine strategies for controlling the problem of seed discoloration associated with this fungus. The survival capability of the pathogen under various storage conditions must be ascertained simultaneously. *L. theobromae* can survive for 12 months in discoloured seeds of bottlegourd and seven months in squash seeds (Maholay and Sohi, 1982) and in soil for at least five months (Aderiye and Ogundana, 1987).

**CONTROL**

**Chemical control**

Many workers have used chemicals to control *L. theobromae* on a variety of seeds or mature plants. Mittal (1983) studied the control of pathogens on forest tree seeds and found the fungicide RH 2161 to be the most effective. For control of microflora on groundnut seeds, Jayanta and Raj (1989) found Dithane M-45 and Emisan 6 to be the most effective fungicides. Demosan, Captan and Brassicol were found by Mohalay and Sohi (1977) to improve the germination of *D. biflorus* seeds which were internally infected with *L. theobromae*. Pigeon pea seeds from field grown plants sprayed either twice or four times, one week apart, with benomyl (2.2 kg/ha) had significantly fewer internally seedborne fungi (Ellis *et al.*, 1977).

Sharma *et al.* (1985) evaluated 15 fungicides against *L. theobromae* in culture and found Bavastin and Tecto to be the most effective against stem (root collar) canker of *Eucalyptus*. They also noted, however, that chemical control on a large scale in the field would not be economically feasible.

Avtar *et al.* (1990) tested six fungicides against *L. theobromae* causing dieback of pear. The most inhibitory to the pathogen in *vitro* were Bavastin and Aureofungin while in the field the best control was given by Blitox-50 followed by carbendazim. For control of *L. theobromae* on post harvest mango fruits, Jagdish and Pathak (1989) found Waxol to be the most effective fungicide. Incidence of postharvest fruit rots of papaya by *L. theobromae* was reduced by
approximately 50% by two-minute dips in a 2-aminobutane carbonated solution at 40 °C and pH 10.5, or by fumigation with 2-aminobutane for two hours at 300 ppm (Hunter 1969). Raghaven and Saksena (1978) did a study to determine the efficiency of fungicides in vitro against some isolates of *L. theobromae*. Of seven fungicides tested Cumen and Vagoll were very effective against 14 isolates. Captan completely inhibited the growth of all but one isolate and Brassicil was effective against most isolates.

Punithalingam (1976) suggests several general control measures against *L. theobromae* including good orchard and plantation hygiene and avoidance of wounding during harvesting and packing. He also lists a group of fungicides effective against the fungus, ie. Basofix B117, Benomyl, Bordeaux 1% oil, Captan, Mancozeb, Dowicide A, Thiophanate and Thiabendazole. Antibiotics such as aureofungin, lagosin and mycostatin and growth regulators such as 2,4-D or 2,4,5-T are also effective against *L. theobromae* (Punithalingam, 1976).

**Biological control**

Various biological control strategies have been used to reduce the impact of diseases caused by *L. theobromae*. Okonkwa *et al.* (1990) isolated *L. theobromae* from banana fruits and controlled infection in the laboratory by manipulating temperature and relative humidity. The fungus ceased rot at room temperature but not at 5 °C or 10 °C. Banana fruit rot caused by *L. theobromae* was inhibited at 90% and 100% RH but not at 10%, 50% and 80% RH.

Florence and Sharma (1990) found *Bacillus subtilis* to be an effective growth inhibitor of *L. theobromae*. Maximum inhibition zones in vitro were 18 mm in diameter. *B. subtilis* has also been found to inhibit conidial germination of *L. theobromae* (Narain and Mohanty, 1984).

Iktun and Adekunle (1990) found that two *Actinomyces* species from soil were more active against *L. theobromae* than *B. cereus* or *B. subtilis*. Soil inoculation of the *Actinomyces* antagonists prevented root and tuber rot of cassava in the field (Iktun and Adekunle, 1990).

**Other methods of control**

Heat treatment of infected plant tissue has been effectively used in the control of various plant pathogens. Hot water treatment of fruits and seeds infected by *L. theobromae* has been reported as an effective control measure. Significant control of postharvest fruit rot of papaya by *L. theobromae* was found by submerging fruits in water at 118 to 120 °F for 20 minutes (Hunter *et al.*, 1969). Blue-stained timber of *Hevea brasiliensis* heated to 75 °C for 24 hours and *Pinus massoniana* heated to 60 °C for 96 hours killed *L. theobromae* (Fu *et al.*, 1988). Gamma-irradiation treatment (75 to 100 krads), alone or in combination with hot water or 2-aminobutane, effectively extended the fruit shelf life (Hunter *et al.*, 1969). *L. theobromae* has been claimed to be effectively eradicated by immersing banana seeds in water at 75 °C for 10 minutes without loss of viability of seeds (Goos *et al.*, 1961).

**CONCLUSION**

*Lasiodiplodia theobromae* is an important pine pathogen and is currently of particular interest to the forestry industry in South Africa. Immediate priorities in research programmes to reduce the impact of *L. theobromae* in local pine plantations should include:

* Studies to determine the time and mode of pine seed infection.
* Chemical, biological or culture control options for reducing seed infection and or pre-emergence damping off.
* An evaluation of inter- and intraspecific resistance of *Pinus* spp. in order to select for more resistant species or clones.

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