# HISTOPATHOLOGICAL OBSERVATIONS ON PINK DISEASE OF EUCALYPTUS

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## ABSTRACT

Host responses of *Eucalyptus grandis* to pink disease were histologically examined. The eucalyptus species are vulnerable to the fungus *Corticium salmonicolor* Berk & Br. The fungus causes cankers and under favourable conditions completely girdle the stem. The stem above the girdle dies due to lack of vertical transport of nutrients and water. The histopathology of the host response reveals that at low levels of infection it resists successfully by obstructing the fungal spread by means of tyloses plugging the vessels in the vicinity and formation of phenolics in the ray parenchyma as well as the axial parenchyma contiguous to the vessels. A deviation from the normal cambial behaviour was also evident.

## INTRODUCTION

*Corticium salamonicolor* Berk & Br. causes pink disease in eucalyptus trees. The pink disease has a worldwide distribution throughout the tropics and subtropics[1–3] and is known to affect more than 141 species including soft woods and hard woods[4]. The defence mechanisms in hard woods have been studied by a number of authors[5–7]. The phenomenon of barrier zone formation as a defence mechanism has been described[8]. It was observed that the starch-containing parenchyma and swollen parenchyma cells function as barrier zones between the infected xylem and the healthy cambium in American elms, and its survival depends upon the pace of barrier zone formation. Such a host response has also been reported in elms affected by Dutch elm disease[9,10]. Shigo and Tippet [11] focussed attention on the phenomenon from the point of view of compartmentalization of decay in trees.

In this paper, we present evidence of tylose formation in the vessels and accumulation of phenolics in the ray and axial parenchyma cells which act as barrier zone against the spread of the fungus resulting in compartmentalization within a small area.

## MATERIALS AND METHODS

Naturally occurring canker specimens of *Eucalyptus grandis* from young and old stems were collected from Meenmutty and Pamba plantations in Kerala state. The samples were fixed in FAA[12] for 2 days and transferred to 70% ethanol. Cross, radial and tangential longitudinal sections of the samples (10 to 20 $\mu$m) were cut with a sledge microtome. The sections were stained with (i) 2% aqueous safaranine and fast green, (ii) 0.5% toluidine blue, and (iii) lactophenol cotton blue schedules. Observations were made on an Olympus microscope with a photomicrographic attachment.

## RESULTS

The cankers are visible dead areas, more or less localized, that develop in the bark and a sapwood regions of the wood. The stem is partially or completely girdled by the fungus under congenial environmental conditions (figure 1). The fungus colonizes the vessels ray and axial parenchyma cells. The spread along the longitudinal axis is mainly through the vessels and radially through the ray parenchyma cells (figure 2). With the onset of infection and spread of the fungus in the tissue, the host responds by the accumulation of dark granular, amorphous material in the axial and ray parenchyma cells to form vertical barriers and radial barrier zones (figures 3–5). The accumulation of these contents in the axial parenchyma cells abutting the vessels was distinct (figure 4). On treatment with ferricchloride these contents turned green indicating their phenolic nature. Tyloses started appearing in the vessels that are in the vicinity of the mycelial front (figures 6 and 7) and gradually increase in number and completely plug the vessels (figure 8). A deviation in the functioning of the cambium in the infection zone was also evident. The cambium was found to produce only fibres towards the centre without any vessels (figure 9).

## DISCUSSION

The fungus penetrates the host tissue and spreads along the axis and radially. Axial parenchyma facilitates a rapid spread due to lack of mechanical resistance. As a response to fungal colonization,

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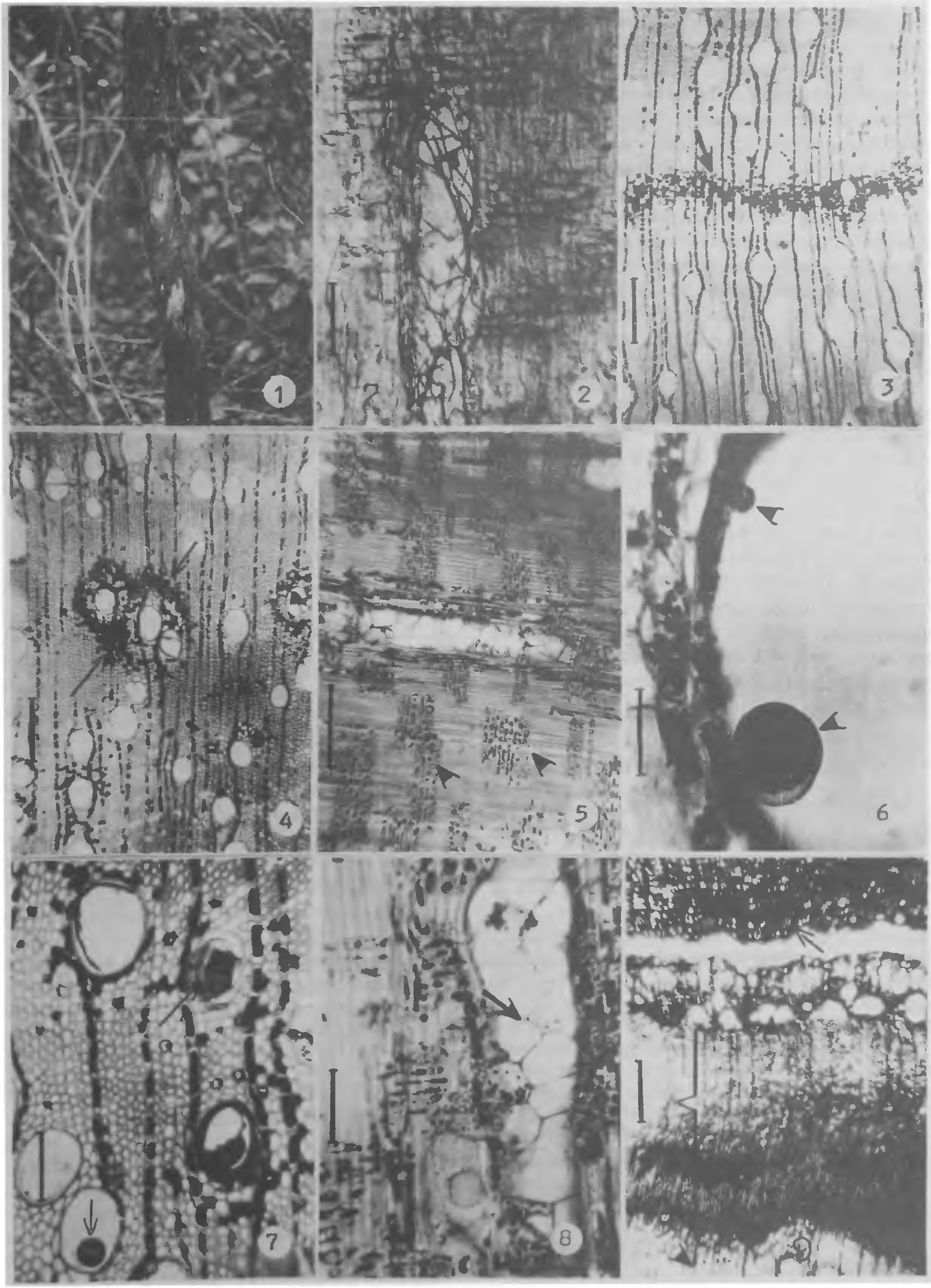
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dark phenolic material accumulates in the ray parenchyma cells surrounding the infected vessels, pointing to a host response to infection as was noticed by earlier workers[13]. Tylose formation in the vessels is abundant and they were often found to be filled with phenolic material. This may hinder the spread of the fungus through the vessels by offering chemi-mechanical resistance. Phenolics are known to be toxic to fungi[7,14]. The accumulation of these toxic compounds may further inhibit the growth of the fungus. In addition the induced tyloses may also prevent the translocation of nutrients to the infected area thus compartmentalizing it. In certain cases the cambial derivatives form exclusively fibres for a period of time and the absence of vessels in this area again diminishes the possibility of vertical spread.

Apparently the rate at which the localization of the infection by the formation of effective barrier zones determines the outcome of the host-pathogen interaction. If the barrier zone formation is rapid and isolates the stored energy reserve and a further infection takes place the amount of energy available to form a fresh barrier becomes limited and the host succumbs. This probably explains why young shoots of trees die so quickly after infection[7]. The thicker branches and trunks survive as the cambium still remains active and form a fresh barrier zone. This corroborates well with the field observations, where beyond three years, the pink disease was not found to be a serious threat.

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**Figures 1–9. 1.** Pink disease affected stem of *Eucalyptus grandis;* **2.** R. L. S. of wood. Note mycelium in vessels, ray and axial parenchyma; **3.** T. S. of the wood in the vicinity of the canker. Arrow indicates tangential band of parenchyma with phenolics; **4.** T. S. of wood. Arrow indicates the vesicentric parenchyma filled with phenolics; **5.** R. L. S. of wood. Arrow heads indicate ray cells filled with phenolics; **6.** T. S. of wood. Arrow heads indicate development of tylose into the vessels; **7.** T. S. of wood. Arrows show tylose filled with phenolics; **8.** R. L. S. of wood showing the vessels packed with tyloses; **9.** T. S. of stem. Arrow points phloem. Arrow heads indicate normal wood and bracket includes anomolous wood cells. Scale: Unit lines indicate figure **1.** 10 cm; **2.** 20 $\mu$m; **7,8.** 20 $\mu$m; **3, 4, 9.** 200 $\mu$m; **5.** 100 $\mu$m; **6.** 10 $\mu$m.