

# Rhizotron: a demonstrative tool for monitoring *in vivo* the infection process of oil palm seedling by *Ganoderma boninense*



Breton, F.<sup>1)</sup>, Hasan, Y.<sup>2)</sup>, Hariadi<sup>2)</sup>, Lubis, Z.<sup>3)</sup>, de Franqueville, H.<sup>1)</sup>

Contact : breton@cirad.fr

- 1) Oil Palm Breeding Research Unit - Tree Crops Department - Cirad
- 2) PTPP London Sumatra - Bah Lias Research Station
- 3) PT Socfindo Medan

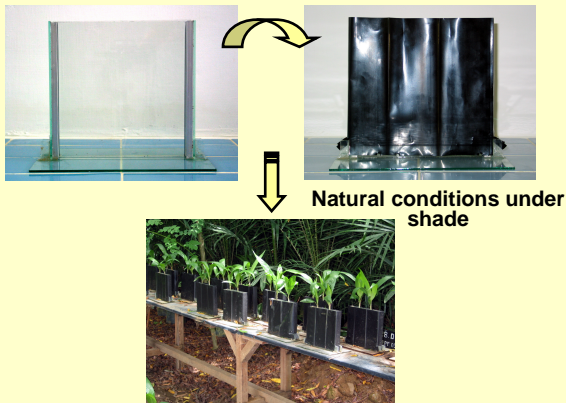
## I - SUMMARY

Rhizotrons allow the monitoring of *Ganoderma* infection, through a clear-walled chamber, after inoculation of germinated seeds with pre-infected substrate. This tool was developed for direct observation *in vivo* of (i) the development of *Ganoderma* isolates on different substrate sources (rubber wood block, rubber sawdust...), (ii) the effect of several treatments (bio-agents, fungicides...) on the pre-infected or infectious substrates tested and (iii) the sampling and analysis of roots at very early stages of infection. Two germinated seeds were inoculated per rhizotron and the seedlings were observed until 6 months after inoculation. The first root symptoms appear two months after inoculation (using pre-infected rubber wood blocks, RWB) while the leaf symptoms are not detected until 4 months after inoculation. The observation of the penetration point demonstrates that a very close contact between roots with pre-infected RWB is required. Penetration of *Ganoderma* mycelium to host tissues seems related to the development of stromatic-like structures surrounding the root on the RWB surface. The induction of stromatic-like structures was also observed without the presence of root contact leading to the hypothesis that there is no strong chemotaxis developed by the fungus. No stromatic-like structure is observed after inoculation using pre-infected rubber sawdust and no infected seedling was obtained. The development of the stromatic-like structures by *Ganoderma* seems to require a solid substrate.

**Keywords :** Rhizotron, *Ganoderma*, oil palm, stromatic-like structure

## II - MATERIALS AND METHODS

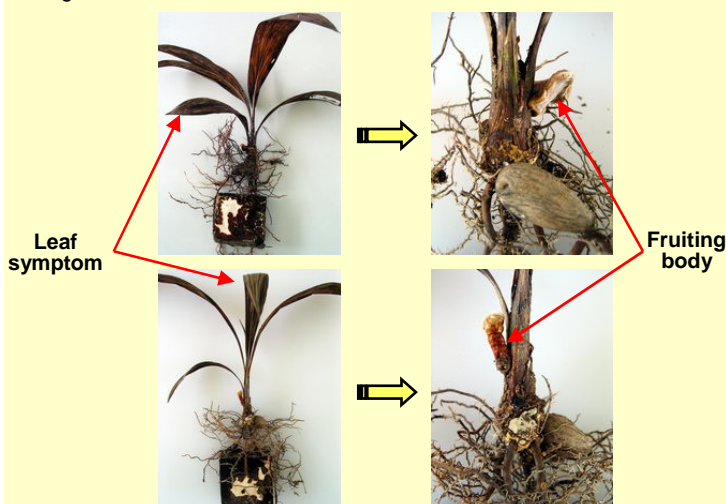
Rhizotron is constituted of two plates of glasses (20x20cm) separated by 3cm. The volume of soil used is 1200cm<sup>3</sup>. Black plastic is used to cover the rhizotron to avoid a light effect and to reproduce natural conditions. Rubber wood blocks (RWBs) and rubber sawdust (RS) were inoculated with an aggressive isolate of *Ganoderma*, then incubated for several weeks in the dark at 27°C. For artificial inoculation of germinated seed, RWB are placed at 8cm from the top of rhizotron. Two germinated seeds were inoculated per rhizotron. Rhizotrons are incubated in natural conditions under shade and are watered twice a day. Seedlings can be conserved in a rhizotron for around 6 months.



## III - RESULTS

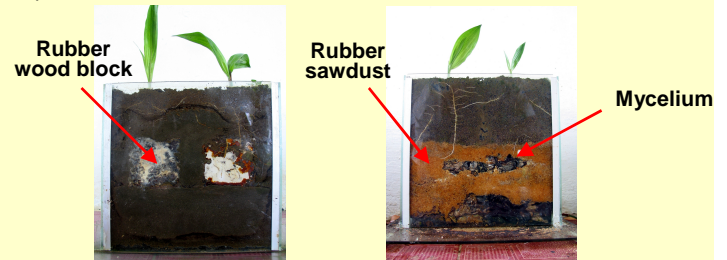
### III-1 Induction of *Ganoderma* disease symptoms

All symptoms of the disease were induced 3-4 months after inoculation of germinated seeds by pre-infected rubber wood blocks. The production of fruiting bodies at the base of the stem have also been observed.

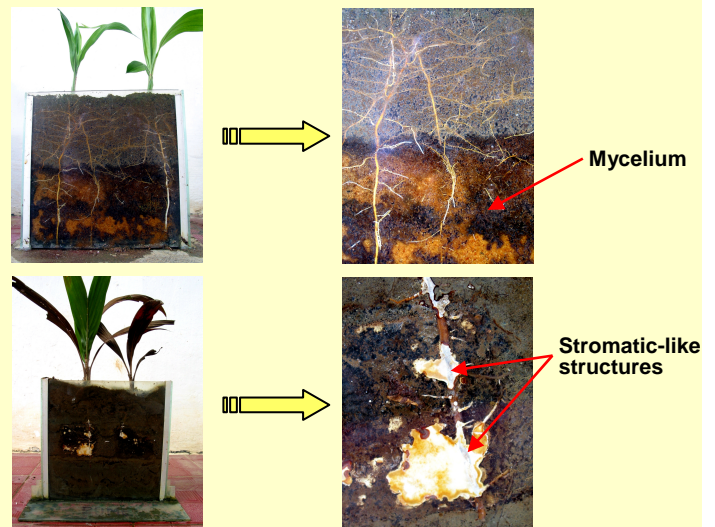


### III-2 Rubber wood block Vs Rubber sawdust

Two pre-infected substrates were tested, RWB (72cm<sup>3</sup>) and RS (300mL), for their capacity to induce Basal Stem Rot disease (BSR) in these experimental conditions.



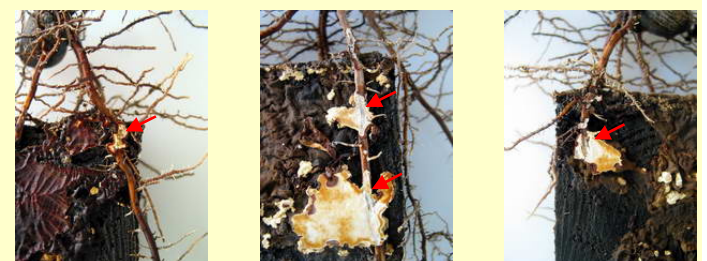
The growth of the roots was observed through the infected RS and in contact with the infected RWBs. During the first months, no difference of seedling and root growth has been recorded in relation to the nursery conditions.



Three to four months after inoculation of germinated seeds, the first leaf symptoms appear with only infected RWBs as inoculum source. Pre-infected RS has not resulted in successful infection of seedlings.

Leaf symptoms were correlated with the production of stromatic-like structure (SLS) by the fungus on the surface of the RWB. No stromatic-like structure was produced with rubber sawdust. The development of these structures seems to require a solid substrate.

These SLS were a very compact knots of mycelium which surrounded the root leading to the penetration of the fungus into the host tissue. No chemotaxis between the production of these mycelium structures with the root contact on the surface of the infected RWBs has been observed. Therefore, the presence of a close contact between the root and the stromatic-like structure seems necessary for the infection of the seedlings by *Ganoderma*.



## IV - DISCUSSION

The rhizotron method has permitted us to improve our knowledge of the infection process of the interaction between oil palm-*Ganoderma* spp.. The infection of the seedling seems to involve a) the production of stromatic-like structure by the fungus on the surface of inoculum source and b) a close contact between the root with this compact structure of mycelium.

This result can lead to some questions :

Is there a relation between the isolate aggressiveness and the production (kinetic...) of the SLS ?

What is the role of these SLS in terms of resistance to antagonistic fungus in the soil (i.e. *Trichoderma*) ?

Is there a relation between the production of SLS with the nature or the degradation process of the inoculum source (or the host tissues) ?