Gas Exchange Responses of Oil Palm to *Ganoderma boninense* Infection

M.H. Haniff, S. Ismail and A.S. Idris
Malaysian Palm Oil Board, P.O. Box 10620, Kuala Lumpur, Malaysia

**Abstract:** A comparison of physiological parameters was carried out between healthy and *Ganoderma* infected 17-year old oil palms grown under the same field conditions. Results from gas exchange measurements indicate that stomatal conductance was significantly reduced in infected palms. This led to significant reductions in transpiration rate and intercellular CO₂ concentration in the infected palms. The relative leaf chlorophyll content and quantum efficiency of PS II were also significantly reduced in the infected palms. The results indicate that infected palms were under water stress that was induced by injury to their root and vascular transport system and not related to soil water deficits.

**Key words:** Oil palm, *Ganoderma boninense*, stomatal conductance, chlorophyll, transpiration, intercellular CO₂, chlorophyll fluorescence, quantum efficiency

**INTRODUCTION**

Basal stem rot in oil palm is caused by *Ganoderma boninense* and it is the most severe fungal disease of oil palm in Malaysia. It has the ability to infect palms from as young as 12-24 months to over 24 years after field planting. High incidences of this disease have been reported in oil palms planted on coastal soil and peat. The incidence of *Ganoderma* in inland soils was relatively low and confined to waterlogged areas. However, Benjamin and Chee reported the presence of disease on palms planted on latereic soils that were previously disease free. Currently, there is no effective treatment for *Ganoderma* infected palms, while preventive measures undertaken show varying degrees of effectiveness. The mode of infection is through the oil palm root system and the disease has reached a critical stage by the time fruiting bodies or foliar symptoms are visible.

Like all fungi, *G. boninense* have no chlorophyll and thus, lack photosynthetic capability. Instead of manufacturing their own food, fungi absorb nutrients from either living or dead host tissue. Thus, parasitic fungi live off the host plant and endanger the host’s health. Many saprophytic fungi can be weakly parasitic in their behavior, especially if their host is dying from other causes. *Ganoderma* is among one of these facultative parasites i.e., a saprophytic fungus activated by favorable conditions to behave parasitically. Carbon assimilates produced by the host palm may be diverted to the sites of infection in response to wounding by the disease. This reduces the supply of carbon assimilates to other developing organs of the host palm, such as new leaves, roots and fruit bunches. *Ganoderma* infection can also affect the absorption and translocation of water and nutrients throughout the host palm. The disruption is due to destruction of the root system, blockage of water and nutrient movement in the xylem or phloem, or the redirection of host nutrients.

In general, plant disease can alter the normal physiology of a plant, which includes many processes such as photosynthesis, respiration, absorption and translocation of water and nutrients, transport of photosynthetic products and production of growth compounds. Some responses to infection occur at the leaf level in response to stimuli generated in the leaf itself or elsewhere in the plant. They have a negative influence on carbon assimilation and growth. However, it is the integrated response at the whole plant level, including carbon assimilation and the allocation of photoassimilates to different plant parts and reproductive ability that finally dictates survival and persistence under environmental stress.

Conditions that reduce the absorption of water and nutrients will have a tendency to impose stress on the palm and affect its ability to carry out photosynthesis. However, when light, nutrients and water are non-limiting, CO₂ availability is more often a limiting factor in the photosynthesis process. Other environment factors that could influence photosynthesis through regulation of stomatal conductance are vapour pressure deficit and temperature. Infected palms are expected to show differences in physiological activity at the leaf level as compared to healthy palms, when grown under the same environmental conditions. This study was undertaken to
investigate the effects of *Ganoderma* infection on gas exchange characteristics of field planted oil palms in Malaysia.

**MATERIALS AND METHODS**

The study was carried out at an experimental field trial at the MPOB Research Station in Kluang, Johor. The field was planted in September 1986 with DxD (Serdang) palms on lateritic soil at 148 palms per hectare and received the standard estate management practice. Palms are considered healthy when they still have intact frond canopy and no fungal fruiting bodies on their trunk, while infected palms had *Ganoderma* fruiting bodies on their trunk. In January 2003, five palms with uniform vegetative appearance (i.e. at least 25 to 30 green fronds per palm) were selected from healthy and infected groups. A 3m scaffolding tower was used to reach the frond leaflets for gas exchange measurements. Measurements were taken from fronds 1, 9 and 17 of each selected palm.

**Gas exchange measurements:** Gas exchange characteristics were measured of 6 upper rank leaflets from the middle region of the chosen frond using a portable photosynthesis system (CIRAS-1, PP-System, UK). The leaf cuvette was controlled at 350 ppm CO₂, 30°C, 70% relative humidity and 1000 µM m⁻² sec⁻¹ Photosynthetically Active Radiation (PAR). Measurements were made between 9 am to 2 pm and completed within 5 days. A pair of healthy and infected palms was measured at each day.

**Relative chlorophyll content and chlorophyll fluorescence measurements:** Relative chlorophyll content of leaves was determined by a chlorophyll meter (SPAD 502, Minolta, Japan). Quantum efficiency of photosystem II (ΦPSII) was determined with a portable chlorophyll fluorescence meter (FMS 2, Hansatech, UK). Measurements were made at the same time as the gas exchange measurements.

**RESULTS AND DISCUSSION**

**Photosynthesis:** Photosynthetic rates measured from fronds of different ages showed no significant difference between healthy and infected palms (Table 1). Both showed decreasing photosynthetic rates with increasing frond age that can be attributed to leaf senescence, position in the canopy or both. Infected palms had a non-significant 13% reduction in mean photosynthetic rate as compared to healthy palms (Table 2). Photosynthesis is limited primarily by light harvesting and assimilatory power under low light and by carboxylation and photorespiration under low CO₂. However, under saturating light and CO₂, photosynthesis may be controlled by processes that convert triose-phosphate into starch and sucrose. Carbon assimilation also decreases as a consequence of limitations to CO₂ diffusion in the leaf, diversion of carbon allocation to non-photosynthetic organs and production of defense molecules, or changes in leaf biochemistry that result in the down regulation of photosynthesis. The photosynthetic rate (A) of both C₃ and C₄ plants also decreases as their relative water content and water potential decrease. In C₃ species such as the oil palm, Rubisco capacity is the primary limitation to A at light saturation and with CO₂ concentrations below the ambient atmospheric value (350 ppm), particularly near the temperature optimum. Photosynthesis rates also decrease with reduction in stomatal conductance.

**Stomatal conductance:** The stomatal conductance (gₛ) was significantly reduced at all frond ages in infected, as compared to healthy palms (Table 1). There was a reduction of about 30.5% in the mean stomatal conductance of the infected palms as compared to healthy palms (Table 2). This implies that the infected palms experience stress that triggers their stomata to partially close. The stress was mainly due to the infection since the palms were under the same environmental conditions. However, the mean stomatal conductance value for the infected palms was still adequate to support gas exchange as indicated by the photosynthetic rates. There was a strong relationship between A and gₛ for both healthy and infected palms (Fig. 1). There is often a close relationship between gₛ and A, because an early and progressive effect of water stress is stomatal closure.
Table 1: Gas exchange, relative leaf chlorophyll content and PS II quantum efficiency of different fronds from healthy and infected palms

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Palm status</th>
<th>Fron 1 No.</th>
<th>Fron 2 No.</th>
<th>Fron 3 No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photosynthesis (μmol CO₂ m⁻² sec⁻¹)</td>
<td>Healthy</td>
<td>7.85±0.52 Aa</td>
<td>6.95±0.70 Aab</td>
<td>6.04±0.34 Ab</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>6.47±0.54 Aa</td>
<td>6.63±0.71 Aab</td>
<td>4.75±0.54 Ab</td>
</tr>
<tr>
<td>Stomatal conductance (mM H₂O m⁻² sec⁻¹)</td>
<td>Healthy</td>
<td>154.48±15.72 Aa</td>
<td>146.73±16.72 Aa</td>
<td>136.43±18.77 Aa</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>95.67±17.10 Ab</td>
<td>108.27±14.30 Bb</td>
<td>98.10±15.88 Ba</td>
</tr>
<tr>
<td>Transpiration (mM H₂O m⁻² sec⁻¹)</td>
<td>Healthy</td>
<td>2.35±0.18 Aa</td>
<td>2.11±0.19 Aa</td>
<td>1.93±0.20 Aa</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>1.52±0.21 Ba</td>
<td>1.76±0.19 Ba</td>
<td>1.56±0.20 Ba</td>
</tr>
<tr>
<td>Intercellular CO₂ (ppm)</td>
<td>Healthy</td>
<td>206.69±3.12 Aa</td>
<td>219.30±5.56 Ab</td>
<td>222.53±6.59 Aa</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>172.24±9.39 Ba</td>
<td>195.77±8.85 Bb</td>
<td>226.83±4.79 Bb</td>
</tr>
<tr>
<td>Relative leaf chlorophyll content (SPAD)</td>
<td>Healthy</td>
<td>63.08±1.81 Aa</td>
<td>69.77±1.19 Ab</td>
<td>71.84±1.31 Ab</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>56.68±1.48 Ba</td>
<td>61.94±1.24 Bb</td>
<td>64.93±1.38 Bb</td>
</tr>
<tr>
<td>PS II quantum efficiency</td>
<td>Healthy</td>
<td>0.76±0.01 Aa</td>
<td>0.72±0.03 Aa</td>
<td>0.72±0.02 Aa</td>
</tr>
<tr>
<td></td>
<td>Infected</td>
<td>0.71±0.01 Ba</td>
<td>0.71±0.01 Ba</td>
<td>0.68±0.02 Ba</td>
</tr>
</tbody>
</table>

Column means±S.E followed by the same capital letter and row means±S.E. followed by the same small letter(s) are not statistically significant at p<0.05.

Table 2: Mean gas exchange parameters measured of healthy and infected palms

<table>
<thead>
<tr>
<th>Palms</th>
<th>Photosynthetic rates (μmol CO₂ m⁻² sec⁻¹)</th>
<th>Stomatal conductance (mM H₂O m⁻² sec⁻¹)</th>
<th>Transpiration rates (mM H₂O m⁻² sec⁻¹)</th>
<th>Intercellular CO₂ (ppm)</th>
<th>C/CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>6.9±0.4a</td>
<td>144.9±9.7a</td>
<td>2.1±0.1a</td>
<td>215.8±5.1a</td>
<td>0.62±0.01a</td>
</tr>
<tr>
<td>Infected</td>
<td>6.0±0.4a</td>
<td>100.7±9.9b</td>
<td>1.6±0.1b</td>
<td>198.3±5.5b</td>
<td>0.57±0.02b</td>
</tr>
<tr>
<td>% reduction</td>
<td>13.0</td>
<td>30.5</td>
<td>23.9</td>
<td>9.1</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Column means±S.E. followed by the same letter are not statistically significant at p<0.05.

Light-saturated stomatal conductances have been used to reflect water stress intensity and are less dependent on the species and other environmental conditions. Many photosynthetic parameters, such as electron transport rate, carboxylation efficiency, intrinsic water-use efficiency and respiration rate in the light are also strongly correlated with stomatal conductance than with water status itself. In grapevines, non-stomatal limitations become important when gs drop below 100-150 mM m⁻² sec⁻¹. Under moderate water deficit, i.e. when photosynthesis is mainly limited by stomatal conductance, a complete recovery of the maximum A occurred one night after irrigation[34,35]. However, when the gs value is less than 50 mM m⁻² sec⁻¹, photosynthesis does not reverse even after irrigation[36]. Further reduction of gs as water stress increases leads to reduced photosynthetic activity.

As water stress progresses, stomatal closure occurs for increasingly longer periods of the day in field-grown plants, beginning in mid-morning[36]. This depression in gas exchange simultaneously reduces daily carbon assimilation and water loss at the time of highest evaporative demand in the atmosphere and leads to a near optimization of carbon assimilation in relation to water supply[37,38]. The causes for this depression in net carbon uptake are still not fully understood and seem to involve mechanisms at both the stomata[39] and chloroplastic level[40].

Transpiration rate (E): Leaf transpiration rate measured in the leaf cuvette was significantly reduced in infected palms by about 23.9% as compared to healthy palms (Table 1 and 2). This can be attributed to the lower stomatal conductance value of the infected palms, which reduces water loss from the leaves. CO₂ assimilation is closely related to leaf transpiration rate through the simultaneous flow of water vapor through the stomata to the atmosphere and CO₂ flow from the atmosphere into the leaf. Leaf transpiration is affected by leaf water content and influence the heat balance, which may influence the photosynthetic rate. The water status of the leaf has a pronounced effect on the stomatal conductance.

Stomatal control of water loss has been identified as an early event in plant responses to water deficit under field conditions, leading to a limitation of carbon uptake by the leaves[41,42]. Stomata close in response to either a decline in leaf turgor, leaf water potential or both[43], or to a low-humidity atmosphere[44,45,46]. Various experiments have shown that stomatal responses are often more closely linked to soil moisture content than to leaf water status. This suggests that stomata are responding to chemical signals, such as abscisic acid (ABA) produced by dehydrating roots, even when leaf water status is kept constant[47,48]. Although most evidence for this kind of response has been obtained under controlled conditions on small plants grown in containers[49,50], experiments with field-grown plants, such as maize[51], grapevines[51,52] and clover[53], also support this hypothesis. Much is known about the role of ABA in closing stomata, as well as its production in dehydrating roots and its circulation in the plant. However, there is still limited knowledge about the exact relationship between water deficit and ABA long distance signaling and the nature of interactions between ABA and other chemical signals, such as cytokinins and ethylenes[54]. In mature trees, where local-distance transport of the chemical signal from the roots to the shoots would
be required, the evidence is even less clear\[16\]. Changes in plant hydraulic conductivity have been thought to play a major role in short-term stomatal regulation of woody plants\[43\]. The interactions between root chemical signaling and changes in plant hydraulic conductivity during drought remain vague and need further consideration\[45\].

**Intercellular CO₂ concentration**: The intercellular CO₂ concentration (Cᵢ) in the infected palms was significantly reduced by about 8.1% compared with healthy palms (Table 2). However, no significant increase in Cᵢ value was observed in frond 17 of the infected palms (Table 1). The higher Cᵢ value of frond 17 was probably caused by the lower stomatal conductance and high leaf respiration rate. Leaf respiration continues after photosynthesis starts to decline since mitochondria remain intact\[46\]. Cᵢ leaves exposed to high temperature often maintain intercellular CO₂ levels that fall on the initial slope of the photosynthetic CO₂ response curve\[42,43\]. This is a region commonly thought to reflect a limitation in rubisco capacity. Rubisco also deactivates at high temperature in C₄ species\[46,47\]. The Cᵢ has also been shown in previous studies to be more dependent on gₑ, than on leaf water potential\[22,24\]. The strong relationship between A and gₑ also indicates that reduction in the transpiration rate and intercellular CO₂ concentration was regulated mostly by stomatal closure.

The ratio of internal to atmospheric CO₂ concentration (Cᵢ/CO₂) in infected palms was reduced by about 8% as compared to healthy palms (Table 2). As stomata close, the CO₂ concentration inside the leaf initially declines with increasing stress and then increases as water stress becomes more severe\[23,30\]. After an early partial closure of stomata, metabolic limitation, caused by either damage (i.e. permanent) or adjustment (i.e. reversible down-regulation) occurs.

**Relative leaf chlorophyll content**: The relative leaf chlorophyll (Chl) content was significantly reduced in the infected palms by about 10.3% as compared to healthy palms (Table 3). It also increased with increasing frond age (Table 1). Chlorophyll is the molecule that absorbs light and uses the energy to synthesize carbohydrates from CO₂ and water. Reductions in leaf Chl content can affect light use efficiency and CO₂ assimilation. Previous research indicates a close correlation between leaf Chl content and leaf nitrogen (N) content. This is because much of leaf N is contained in Chl\[49\]. Both decreased and unchanged Chl levels during drought stress have been observed in other plant species, depending on drought duration and severity\[18-52]\.

Plants subjected to water shortage can have accelerated leaf senescence because it reduces the water demand and allows recycling of scarce resources to the reproductive sink\[35\]. During leaf senescence, a large part of the leaf nitrogen, carbon and minerals is recycled to other organs of the plant\[34\]. However, early leaf senescence will reduce crop yield because cumulative photosynthesis is reduced\[36-59\].

**Quantum efficiency PS II**: Quantum efficiency of PS II (Φₚₛₛ) was significantly reduced in the infected palms by about 4.1% as compared to healthy palms (Table 2). It also tended to decrease with increasing frond age (Table 1). The lower Φₚₛₛ value in the infected palms implies that their light use efficiency was affected, since the fluorescence measurement reflects changes in the efficiency with which absorbed light is used for PS II photochemistry. In healthy leaves, this value is always close to 0.8, independently of the plant species studied. A lower value indicates that a proportion of PS II reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants under stress conditions\[57,58\]. Chlorophyll fluorescence also showed a high dependency on stomatal conductance.

In perennial crops such as the oil palm, the dissipation of excitation energy at the chloroplast level through processes other than photosynthetic carbon-metabolism is an important defense mechanism under conditions of water stress and is accompanied by down-regulation of photochemistry and, in the longer term, of photosynthetic capacity and growth.

When water deficit develops slowly, one of the first events to take place in plants is stomatal closure in response to the migration of chemical compounds synthesized in dehydrating roots (e.g. ABA). The decline in intercellular CO₂ following stomatal closure and the lower light use efficiency under drought may induce, in the long-term, a down-regulation of the photosynthetic machinery to match the available carbon substrate.

**Ganoderma** infection strongly affected the leaf gas exchange of oil palms through a reduction in stomatal conductance, which led to a significant reduction in transpiration rates and intercellular CO₂ levels. Infected palms had lower relative leaf chlorophyll content and lower quantum efficiency of PS II. These responses imply that infected palms were subjected to water stress as a
result of *Ganoderma* induced injury to the root and vascular transport systems. The stress was not related to soil water deficits since the palms were grown under the same environmental conditions. This information is useful for developing a suitable method for early detection of *Ganoderma* infection in oil palm. Further research is needed to ascertain the physiological mechanisms involved, particularly during the initial stage of infection.

**ACKNOWLEDGMENTS**

We wish to thank the Director General of MPOB for permission to publish this work, Mr. Maurad Ahmad and Mr. Abdullah Badrishah for technical assistance, Mr. Zaki Aman for logistic support and Dr. I.E. Henson, Dr. Ariffin Darus and Dr. Norman Kamaruddin for useful comments on the manuscript.

**REFERENCES**


