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Influence of Infection by Corynespora cassiicola *on Carbon Dioxide Assimilation Rate in* Hevea *Leaves*

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Leaf infection by Corynespora cassiicola *has been detected in a number of* Hevea *clones grown in Sri Lanka. The CO_2 assimilation rates and related parameters of apparently healthy tissues in infected leaves of highly susceptible, moderately susceptible and mildly susceptible* Hevea *clones were studied. The CO_2 assimilation rates were lower in apparently healthy tissues of infected leaves. The reduction was more marked in susceptible clones. There was no difference in the chlorophyll content in healthy leaves and in apparently healthy tissues of infected leaves used for measuring gas-exchange parameters. The changes in stomatal conductances, internal CO_2 concentration and apparent quantum efficiency (CO_2) suggested that lowering of CO_2 assimilation rates was due to changes in the photosynthetic mechanism of infected leaves. Toxic compounds liberated by the fungus might bring about such changes in the photosynthetic mechanism. The productivity in highly susceptible clones was affected by lower conversion efficiencies of the infected leaves and light interception capacities of the canopies.*

Corynespora leaf fall (CLF) disease caused by *Corynespora cassiicola* (Berk and Curt) wei was detected in Sri Lanka for the first time in the later part of 1985 on *Hevea brasiliensis* Muell Arg[1]. A few clones were susceptible while the others showed varying degrees of tolerance to the disease. Immature copper-brown leaves and semi-mature apple-green leaves were highly susceptible. In diseased leaves, the area around the lesion gradually became chlorotic. In highly susceptible clones, defoliation resulted, presumably due to the presence of a toxic substance liberated by the fungus[2]. Repeated defoliation due to the disease caused a severe retardation of growth, ultimately causing die-back of shoots and branches or even death of trees. This resulted in serious losses in the yield of rubber.

The CO_2 assimilation rates (conversion efficiency — A) of infected leaves have been studied for various crops. Pea *(Pisum sativam)* leaves infected with powdery mildew fungus *(Erysiphe pisi)* had CO_2 assimilation rates below that found in healthy leaves within 24 h of inoculation[3]. Leaves of oak *(Quercus robur)* seedlings infected with powdery mildew fungus *(Microsphaera alphitoides)* showed an initial increase in CO_2 assimilation rates, followed by a rapid decline to levels significantly lower than that of control leaves[4]. CO_2 assimilation rates were stimulated in third seedling leaves of barley *(Hordeum vulgare)* plants whose lower leaves were heavily infected with powdery mildew *(Erysiphe graminis)*[5]. The differences in CO_2 assimilation rates between healthy and infected leaves were attributed to changes in biochemical aspects[4], stomatal conductances[3] and respiratory rates[3,5].

The retardation of growth and the yield losses in infected *Hevea* clones suggested that the productivity had declined. Total productivity of a plant is determined by the products of total light, interception efficiency and conversion efficiency. How the CLF disease influenced the latter two were studied for three *Hevea* clones *viz.*, RRIC 103, RRIC 52 and RRIM 600 categorised as highly susceptible, moderately susceptible and mildly susceptible, respectively.

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CO_2 assimilation rates *(A)*, stomatal conductances *(gS)*, internal leaf CO_2 concentrations *(Ci)* and transpiration rates *(E)* were measured simultaneously to find out the possible reasons for any changes in *A*.

MATERIALS AND METHODS

Gas-exchange parameters of apparently healthy regions in infected leaves were compared with those of healthy leaves of the same age. The infection of the leaf was quantified by calculating the percentage infected area of the leaf. The differences in *A* between infected and healthy leaves were expressed as percentages of that parameter in healthy leaves. An eight-year-old RRIC 103 clearing and a year-old nursery raised from budded stumps having clones RRIC 52 and RRIM 600 were used to obtain leaf material for the study.

Gas-exchange Measurements

A laboratory system based on an Infra-red Gas Analyser — IRGA (ADC Ltd., Type 225 MK II) and two Lee — Integer Humidity/ Temperature probes (Modle DHL 40) were used for simultaneous measurements of CO_2 and water vapour differentials. Leaf sections taken from detached leaves were placed in a leaf section chamber (ADC Ltd., UK) for measurements. A volpi lamp (Modle 250 H) was used as the light source and the light level was maintained at 1200 μ $Em^{-2}s^{-1}$. Light levels were changed for measuring apparent quantum efficiency, by using neutral density filters. Photo-respiration rates were estimated by measuring the post-illumination CO_2 outburst. Water was circulated (Techne Modle C - 400) through the leaf chamber to control its temperature. A Comark (model 2001) electronic thermometer and a thermocouple were used to measure leaf surface temperature. Gas-exchange parameters *viz.*, CO_2 assimilation rates *(A)* photo-respiration rates *(Rp)*, stomatal conductances *(gS)*, internal CO_2 concentrations *(Ci)*, transpiration rates *(E)* and apparent quantum efficiency (CO_2) were calculated as described by Long and Hallgren[6].

Chlorophyll Determination

Chlorophyll contents in healthy leaves and in apparently healthy tissues of infected leaves were determined[7]. Chlorophyll contents of tissues around the lesion were also determined.

Light Interception

A healthy clearing (RRIC 110) and an infected clearing (RRIC 103) of the same age and situated near to each other were selected. Light measurements were done at forty random points within each canopy. Simultaneously, the amount of light was measured in a clear area near the clearings concerned to calculate the light intercepted. Two Li-Cor quantum sensors were used for measuring light intensity.

RESULTS

The value of *A* of infected leaves was always lower than that of healthy leaves. Leaves of clone RRIC 103 (highly susceptible to *Corynespora*) showed a marked drop in *A* even with a slight infection *(Table 1* and *Figure 1)*. In RRIM 600 (a mildly susceptible clone) the drop in *A* was marked only when about 50% or more of the leaf was infected *(Table 2)*. In RRIC 52, the drop in *A* due to Corynespora infection was higher than that in RRIM 600, but lower than that in RRIC 103 *(Figure 1* and *Table 3)*.

A slight infection (10% - 30%) caused the *gS* to decrease significantly in RRIC 103 *(Table 3)*. In clones RRIC 52 and RRIM 600, the drop in *gS* with increasing infection was not significant *(Tables 2* and *3)*.

The value of *Ci* increases with increasing degree of infection. The increase is significant when infection is 30%–50% or more in clone RRIC 103 and when it is 50%–70% or more in clone RRIC 52. The increase in *Ci* in infected leaves, is not significant in clone RRIM 600 *(Tables 1, 2* and *3)*.

The differences in photo-respiration rates, between healthy and infected leaves, are not significant in all three clones *(Tables 1, 2* and *3)*. Anyhow in comparison to their CO_2

TABLE 1. INFLUENCE OF CORYNESPORA INFECTION ON GAS-EXCHANGE PARAMETERS OF CLONE RRIC 103

Infection (%)	Parameter					
	A (μmol $m^{-2} s^{-1}$)	gS (μmol $m^{-2} s^{-1}$)	Ci (μmol mol^{-1})	ϕCO_2	E (mol $m^{-2} s^{-1}$)	Rp (μmol $m^{-1} s^{-1}$)
0 (Healthy)	5.24	0.191	285	0.0146	0.0042	3.0188
10 — 30	*2.29*	*0.153*	*294*	*0.0051*	*0.0043*	*2.8705*
30 — 50	2.29	0.143	314	0.0041	0.0031	2.6853
50 — 70	2.57	0.132	312	0.0047	0.0033	2.6802
Significance of treatment difference	**	*	**	***	*	N.S.
L.S.D. (5%)	1.12	0.028	11.8	0.0036	0.0006	

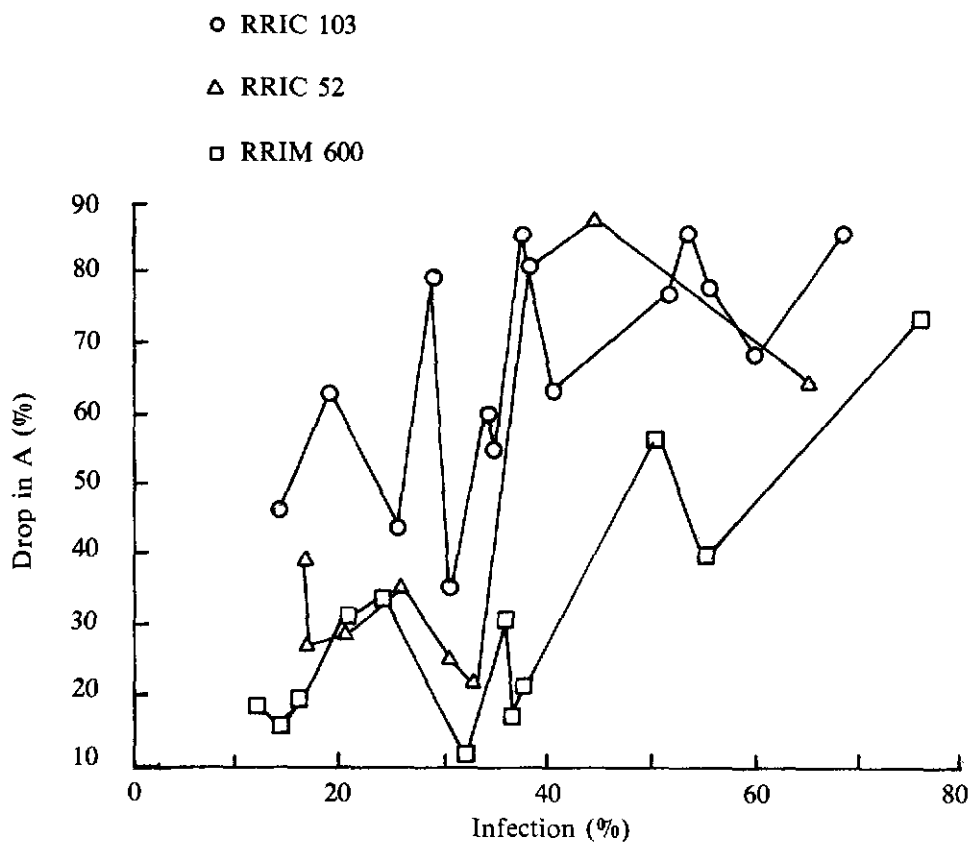

Figure 1. Influence of Corynespora leaf infection on CO_2 assimilation rates (A).

TABLE 2. INFLUENCE OF CORYNESPORA INFECTION ON GAS-EXCHANGE PARAMETERS OF CLONE RRIM 600

Infection (%)	Parameter					
	A (μmol $m^{-2}s^{-1}$)	gS (μmol $m^{-2}s^{-1}$)	Ci (μmol mol^{-1})	ϕ CO_2	E (mol $m^{-2}s^{-1}$)	Rp (μmol $m^{-1}s^{-1}$)
0 (Healthy)	14.3	0.3007	252	0.0244	0.0065	2.47
10 — 30	13.2	0.2775	250	0.0193	0.0063	2.64
30 — 50	11.8	0.2501	255	0.0179	0.0060	2.64
50 — 70	7.95	0.2138	276	0.0120	0.0047	3.48
Significance of treatment difference	*	N.S.	N.S.	N.S.	N.S.	N.S.
L.S.D. (5%)	2.62					

TABLE 3. INFLUENCE OF CORYNESPORA INFECTION ON GAS-EXCHANGE PARAMETERS OF CLONE RRIC 52

Infection (%)	Parameter					
	A (μmol $m^{-2}s^{-1}$)	gS (μmol $m^{-2}s^{-1}$)	Ci (μmol mol^{-1})	ϕ CO_2	E (mol $m^{-2}s^{-1}$)	Rp (μmol $m^{-1}s^{-1}$)
0 (Healthy)	12.60	0.2962	263	0.0175	0.0058	2.9911
10 — 30	8.29	0.2739	274	0.0107	0.0058	3.6050
30 — 50	8.99	0.2473	278	0.0096	0.0052	3.0882
50 — 70	3.84	0.1846	305	0.0052	0.0038	4.3475
Significance of treatment difference	*	N.S.	*	*	*	N.S.
L.S.D. (5%)	3.48		20.3	0.0006	0.0009	

assimilation rates, the photo-respiratory rates are high in infected leaves.

There is no difference in the chlorophyll content in healthy leaves and apparently healthy tissues of infected leaves *(Table 4)*. However, tissues surrounding the lesion had a significantly lower chlorophyll content *(Table 5)*.

Light interception by the canopy is 50.7% and 66.5% less in five- and six-year-old RRIC 103 clearings infected with *Corynespora* respectively *(Table 6)*.

DISCUSSION

It is well documented that foliar infections result in lowering of their CO_2 assimilation rates. The measurements have been done using the entire leaf, including healthy and infected tissues. CO_2 assimilation rates and related parameters of apparently healthy tissues in *Corynespora* infected *Hevea* leaves were compared with those of healthy leaves of the same age in this study.

The CO_2 assimilation rates in the healthy tissues of infected leaves are less affected in

TABLE 4. CHLOROPHYLL CONTENT IN HEALTHY LEAVES AND APPARENTLY HEALTHY TISSUES OF INFECTED LEAVES IN CLONES RRIC 103, RRIC 52 AND RRIM 600

Clone	Chlorophyll content (μ mol cm^{-2})			
	0 (Healthy)	10% - 30% infection	30% - 50% infection	50% - 70% infection
RRIC 103	3.21	2.94	3.03	2.61
RRIC 52	3.71	3.25	3.11	—
RRIM 600	3.86	3.22	2.83	3.69

TABLE 5. CHLOROPHYLL CONTENT IN HEALTHY TISSUES AND TISSUES SURROUNDING THE LESION

Clone	Chlorophyll content (μ mol cm^{-2})	
	Healthy	Infected
RRIC 103	5.31	3.80
RRIM 600	5.32	1.92
Significance of treatment difference	*	
L.S.D. (5%)	*1.06*	

TABLE 6. LIGHT INTERCEPTION BY HEALTHY (RRIC 110) AND CORYNESPORA *INFECTED CANOPIES (RRIC 103)*

Age of clearing (year)	Light interception (%)		Drop in interception (%)
	Infected	Healthy	
5	43.8	89.0	50.7
6	32.6	97.4	66.5

the mildly susceptible clone RRIM 600. In susceptible clones, a marked drop in CO_2 assimilated rate was observed even with a slight infection *(Figure 1)*. Some workers[5] have reported that CO_2 assimilation rates are stimulated in healthy leaves of diseased plants. Stimulation of CO_2 assimilation rates of tissues around the infection site in the early stages of foliar infection has also been reported[4].

The drop in CO_2 assimilation rates observed in infected *Hevea* leaves could be due to either a decrease in *gS* or damage to the photosynthetic mechanism. Though *gS* is low in infected leaves, it is significant only in the highly susceptible clone RRIC 103 *(Table 1)*.

The increase in *Ci* is significant in highly and moderately susceptible clones *i.e.* RRIC 103 and RRIC 52 *(Tables 1, 2* and *3)*. The increase in *Ci*, together with a decrease in *gS* suggest that CO_2 entering the leaf is not being utilised for photosynthesis. Hence, the drop in *A* in infected leaves is more likely to be caused by damage to the photosynthetic mechanism. The significantly lower apparent quantum efficiency (CO_2) in the infected leaves of susceptible clones provides further evidence for this.

There is no drop in the chlorophyll content in the apparently healthy tissues of an infected leaf used for measuring *A*. Hence, the decrease in CO_2 assimilation rate is not due to destruction of the chloroplasts. Tissues around the lesion had significantly lower chlorophyll content.

The decline in CO_2 assimilation rates in infected leaves could be because of biochemical changes[4]. The lower stomatal conductances could bring about such changes in net photosynthesis[3]. Results of this study suggest that stomatal closure resulting in lowering of *gS* could be a secondary effect of high *Ci*.

The significant lowering of *E* in the two susceptible clones could be due to lowering of *gS*. Increases in *E* due to foliar infections of barley[8] and oak[4] leaves have also been reported. The extra water loss is thought to have taken

place from the surface of the fungus on the infected tissues.

Some researchers have attributed the decrease in CO_2 assimilation rates to changes in respiratory metabolism[3,5]. In this study there was no evidence for a significant increase in the respiratory metabolism of infected leaves. Anyhow in comparison to their CO_2 assimilation rates, the photo-respiratory losses were high in infected leaves.

In susceptible clones, under conditions favourable for CLF disease, leaf fall could occur resulting in lowering of light interception by the canopy. The light interception by the canopy of five- and six-year-old infected RRIC 103 clearings was compared to that of healthy canopies of the same age (clone RRIC 110). It was 51% and 67% less respectively, in the infected canopies. Hence, in clones highly susceptible to CLF disease, the productivity is decreased by lower conversion efficiencies of the infected leaves and light interception capacities of the canopies.

It is evident that infection by *Corynespora* lowers the CO_2 assimilation capacity of the apparently healthy tissues of an infected leaf. The decline of CO_2 assimilation capacity may be attributed to an influence on the photosynthetic mechanism. Toxic compounds liberated by the fungus[2], might be responsible for this. This decline in CO_2 assimilation capacity is more marked in highly susceptible clones and is shown even with a slight infection. This together with comparatively high respiratory losses could result in infected leaves of these clones becoming more vulnerable to leaf fall. In mildly susceptible clones, the CO_2 assimilation rate in apparently healthy tissues is less affected and leaf fall would not occur unless the infection is severe.

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REFERENCES

1. LIYANAGE, A.de S., JAYASINGHE, C.K., LIYANAGE, N.I.S. AND JAYARATNE, A.H.R. (1986) *Corynespora* Leaf Spot Disease of Rubber *(Hevea brasiliensis)* — A New Record. *J. Rubb. Res. Inst. Sri Lanka,* **65,** 47.

2. LIYANAGE, N.I.S. AND LIYANAGE, A.de S. (1986) A Study on the Production of a Toxin in *Corynespora cassiicola. J. Rubb. Res. Inst. Sri Lanka,* **65,** 51.

3. AYRES, P.G. (1976) Patterns of Stomatal Behaviour. Transpiration and CO_2 Exchange in Pea following Infection by Powdery Mildew *(Erysiphe pisi). J. Expt. Bot.* **27 (101),** 1196.

4. HEWITT, H.G. AND AYRES, P.G. (1975) Changes in CO_2 and Water Vapour Exchange Rates in Leaves of *Quercus robur* infected by *Microsphaera alphitoides* (Powdery Mildew). *Physiol. Pl. Path.,* **7,** 127.

5. WILLIAMS, G.M. AND AYRES, P.G. (1981) Effects of Powdery Mildew and Water Stress on CO_2 Exchange in Uninfected Leaves of Barley. *Pl. Physiol.,* **68,** 527.

6. LONG, S.P. AND HALLGREN, J.E. (1986) Measurement of CO_2 Assimilation by Plants in the Field and Laboratory. *Techniques in Bioproductivity and Photosynthesis. (Coombs, J., Hall, D.O., Long, S.P. and Scurlock, J.M.O. ed.),* U.K.: Pergamon Press.

7. COOMBS, J., HIND, R.C. AND LEEGOOD, L.L. (1986) Analytical Techniques, *Techniques in Bioproductivity and Photosynthesis (Coombs, J., Hall, D.O., Long, S.P. and Scurlock, J.M.O. ed.).* U.K.: Pergamon Press.

8. MAJERNIK, O. (1965) Water Balance Changes of Barley infected by *Erysiphe gramins. Phytopathologische Zeitscgruft,* **53,** 145.