CHARACTERIZATION OF PHOTOSYNTHETIC APPARATUS OF *Brasiliorchis porphyrostele* (ORCHIDACEAE) INFESTED BY MEALYBUG (*Diaspis boisduvalii*) USING CHL *a* FLUORESCENCE OLKJIP TRANSIENT MEASUREMENTS

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ABSTRACT: The study presents the impact of mealybug [*Diaspis boisduvalii* (Signoret, 1869)] attacks on the "vitality" of *Brasiliorchis porphyrostele* (Orchidaceae) using the polyphasic Chl a fluorescence transient (OJIP) analyses, obtained using a portable fluorometer Handy-PEA (Hansatech, UK). Mealybug infestations were detected in 50% of the plants (plants without visible symptoms were used as controls). Infested plants failed to show a typical polyphasic Chl a fluorescence OJIP. The rise at the O-step and a large depression at the P-step, reductions at photochemical phase O-J and thermal phases J-I and I-P, positive ΔL, ΔK, ΔJ and ΔI steps were observed under infestation, which evidenced a positive K-band and L-band as well as a positive behavior between O-I steps and negative between I-P steps evaluated through the curve WOI. Mealybug infestation decreased all yields parameters and the reductional fluxes of the PSI end electron acceptors, resulting in damages on all photochemical and non-photochemical redox reactions. Decreases in energetic connection and damages on OEC were observed on leaves infested. Negative amplitudes of ΔI and ΔWIP suggest higher damages on PSII photochemistry compared to PSI.

Index terms: biotic stress; energetic connectivity; energy flux; orchidaceae neotropical; oxygen evolving complex; photosystem II photochemistry

CARACTERIZAÇÃO DO APARATO FOTOSSINTÉTICO DE *Brasiliorchis porphyrostele* (ORCHIDACEAE) INFESTADA POR COCHONILHA (*Diaspis boisduvalii*) USANDO MEDIDAS DA FLUORESCÊNCIA TRANSIENTE DA CLOROFILA *a*

RESUMO: Este estudo apresenta o impacto do ataque de cochonilhas [*Diaspis boisduvalii* (Signoret, 1869)] sobre a "vitalidade" de *Brasiliorchis porphyrostele* (Orchidaceae) usando análises da fluorescência transiente da clorofila a (OJIP), obtidas usando-se um fluorômetro portátil Handy-PEA (Hansatech, UK). Infestações por cochonilhas foram detectadas em 50% das plantas (plantas sem sintomas visíveis foram usadas como controle). As curvas polifásicas OJIP foram atípicas nas plantas infestadas. O aumento do ponto O e a expressiva depressão do ponto P, seguidos da redução das fases fotoquímicas O-J e termais J-I e I-P, além de ΔL, ΔK, ΔJ e ΔI positivos, foram observados nas plantas sob infestação, as quais evidenciaram bandas K e L bem como um comportamento positivo entre os pontos O-I e negativo entre os pontos I-P, avaliados através da curva WOI. A infestação por cochonilhas reduziu todos os parâmetros de produção quântica e os fluxos de redução do fotossistema I e aceptores de elétrons, resultando em danos sobre todas as reações redox fotoquímicas e não-fotoquímicas. Reduções na conectividade energética e danos no CEO foram observados nas folhas infestadas. Amplitudes negativas de ΔI e ΔWIP sugerem maiores danos sobre a fotoquímica do FSI comparada à do FSI.

Termos para indexação: estresse biótico; conectividade energética; fluxo energético; orchidaceae neotropical; complexo de evolução do oxigênio; fotossistema II, fotoquímica.

INTRODUCTION

Mealybugs are serious pests of orchids in homes and greenhouses and they are not particular about their host and probably all species of orchids are susceptible to attack of mealybugs, mainly when cultivate. Under mealybug infestation, decreases of plant growth and of number/size of inflorescences, loss of vigor, a weakening and loss of leaves and buds and yellowing leaves with subsequent chlorotic patches and leaf deformations (Chia & He, 1999) are frequently observed. Most commonly, responses of plants to biotic stress also include closure of stomata followed of reduction in CO_2 assimilation rate and partial or total disorganization of photosynthetic complexes.

Recent studies have been indicated that the PSII is more stable (Adamski et al., 2011) compared to PSI under stress conditions. Stability is defined here as the capacity to resist or the degree of resistance to change or disintegration. Thus, an environmental factor that changes the behavior of PSII should also affect the stability of then. To evaluate the stability of PSII under several environmental conditions, chlorophyll *a* fluorescence measurements have been used giving us informations on the relationship between it structure and function and core complexes related (Strasser et al., 2004). The manner which the stress affects the structure and function of PSII and, consequently, their stability, can vary and differences between species are frequently under dependence of type, intensity and exposure time of stress.

It is general consensus that the activity of oxygen-evolving complex (OEC) associated to donator side of PSII is highly impaired by stress, while the effects on PSI activity is still very contradictory. The activity of OEC is evaluated through K-band analyse. The appearance of K-band, which occurs at ~300 μs of illumination, is very common under environmental stresses (Srivastava et al., 1997) and is associated with disequilibrium between the electron donor and acceptor sides of PSII (Strasser, 1997). Generally, disassociations of OEC that use manganese (Mn) as a cofactor is related in this conditions, impairing the improving of electrons to reaction center associated with PSII. Also, the degree of connectivity or grouping between active PSII units or the organization of the membrane structure of the thylakoids is also correlated with the difference in stability between photosystems (Strasser & Stirbet, 1998). The analyses of L-band prove us informations about the connectivity of PSII units, which should assume positive values, indicating lower energetic connectivity (Yusuf et al., 2010). According to Adamski et al. (2011), a positive L-band suggests increases of dissipation, which should be a mechanism to improve the use of excitation energy. Thus, the analyse of L and K-bands should be a useful tool to identify differences in stability between photosystems in plants submitted to stress.

Therefore, contrasting results have been described in the literature, revealing no effects on PSI activity in plants growing under environmental stress. For example, effects of salt stress in cyanobacterium *Spirulina platensis* showed a decrease in PSII mediated oxygen evolution activity while an increase in PSI activity was observed (Sudhir et al., 2005). Lin et al. (2009) evaluated the IP phase of OJIP chlorophyll fluorescence, which is correlated with the redox state end acceptors at PS I electron acceptor side and observed a decreased maximum amplitude of IP phase. This result, when associated with those observed to J-step and I-step, suggest that acceptor side of PSII become more reduced under phosphorus deficiency, but the acceptor side of PSI become more oxidized. In sweet potato (*Ipomoea batatas* L.) growing under conditions of excess iron (Fe), the Fe-induced stress affected the process involving the capture of the exciton to the reduction of PQ, but the electron flux from PQH_2 toward the final electron acceptor does not affected, such as revealed through analyzing of I-P phase after normalizations procedure (Adamski et al., 2011). Taken together, these results confirm that the stability of PSII is dependent on type of stress imposed. No study has yet been conducted to characterize the effects of infestation by mealybug on stability of photosynthetic apparatus of infested orchid plants as well to identify the specific action site of infestation. In this study, we characterized the photosynthetic apparatus of *B. porphyrostele* orchid plants infested by

mealybug in order to test the following hypothesis: (1) damages on OEC, which should be evidenced by significant increase of initial fluorescence rise (F_o) and positive amplitude of K-band related to those parameters describing photoinibition (DI_o/RC and ET_o/RC) show us that the reductional side of PSII is more impaired than acceptor side of PSII under mealybug infestation (Force et al., 2003; Dias & Marenco, 2006) and (2) the conversion of active reaction centre in non-Q_A-reducing centre (heat sinks) and the relative stability of redox reactions of ferredoxin-Fd, other intermediates and NADP is reflected in higher damages on PSII acceptor side (Strasser et al., 2004).

MATERIALS AND METHODS

***Plant materials and growth conditions*:** plants of *B. porphyrostele* were obtained from a private nursery (São Mateus, Espírito Santo State, Brazil) and were cultivated under PPFD was 400-600μmol $m^{-2}s^{-1}$ and temperature ranging from 24 to 30°C. The plants were watered daily and fertilized twice weekly with a nitrate-based commercial fertilizer (N : P : K ratio of 1 : 1 : 1). About 50% of the plants presented symptoms of mealybug infestation. Plants without any visible symptoms were used as controls. All measurements were made on the third leaf from the top (counting down from the youngest mature leaf). The leaves were dark-adapted for 30 min using a leaf clip before measurements were made (*Hansatech*).

***Chl a transient fluorescence*:** polyphasic Chl *a* fluorescence transients (OLKJIP) were measured with a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd., King's Lynn, Norfolk). The transients were induced by 1s illumination with an array of six light-emitting diodes providing a maximum light intensity of 3000μmol (photons)$m^{-2}s^{-1}$. The fast fluorescence kinetics (F_o to F_m) was recorded from 10μs to 1s and used to calculate the parameters of JIP-test (Strasser & Strasser, 1995).

Statistics: *t*he Chl *a* fluorescence polyphasic fluorescence rise (OJIP) was obtained using the Biolyser program (Biolyser © R. M. Rodriguez, The Bionenergetics Laboratory, University of Geneva, Geneva, Switzerland) and plotted on a logarithmic time scale from 50 μs to 1 s. The JIP-test parameters obtained on different leaves with the same level of infestation were analyzed by variance analysis (ANOVA) using SAEG version 4.0 software ($p \leq 0.05$) (Table 1).

RESULTS AND DISCUSSION

The OJIP transients showed a typical polyphasic with the fluorescence signal rising from the initial fluorescence level (F_o) to the maximal level (F_m) with well-defined intermediate J and I steps, demonstrating that all samples were photosynthetically active (Fig. 1*A*). OJIP transient of infested leaves showed a rise at the O-step and a large depression at the P-step (Fig. 1*A*). Our results also showed that mealybug infestation resulted in an increase in the heterogeneity of samples (data not show). The JIP-steps were much higher in non-infested *B. porphyrostele* plants (Fig. 1*A*), and the time required to reach F_m was 209ms and 304ms in non-infested and infested plants, respectively. As such, mealybug infestation caused an increase in the time needed to reach the P-step in the polyphasic Chl *a* fluorescence transient in *B. porphyrostele* plants. Additionally, the mealybug infestation reduced the area beneath the fluorescence curve between F_o and F_m. Our results also showed reductions at photochemical phase O-J and thermal phases J-I and I-P under mealybug infestation, with higher effects on I-P phase, which was much more reduced by mealybug infestation (Fig. 1*A*).

In order to compare the effects of mealybug infestation for the events reflected in the O-J, J-I and I-P phases, the fluorescence data were double normalized (between F_o and F_m) and presented as the kinetics of relative variable fluorescence at any time, $V_t = (F_t - F_o)/(F_m-F_o)$, and as a difference kinetic profile ΔV_t ($Vt_{treatment}$ - $Vt_{control}$) (Strasser et al. 2007). As shown in Fig. 1*B*, mealybug infestation displayed positive ΔL, ΔK, ΔJ and ΔI steps compared with controls around 130μs, 300μs, 2ms and 30ms, respectively and decreased the maximum amplitude of I-P phase.

Therefore, to must clearly show the effects of infestation by mealybug on structure and functionality of photosynthetic apparatus

in *B. porphyrostele*, the fluorescence data were double normalized between the steps O-J [as $W_{OJ} = (F_t-F_o)/(F_J-F_o)$] and between J-P [as $W_{OP} = (F_t-F_J)/(F_m-F_J)$] and plotted with the difference kinetics $\Delta W_{OJ} = W_{OJ(treatment)} - W_{OJ(control)}$ (Strasser et al., 2007) and $\Delta W_{JP} = W_{JP(treatment)} - W_{JP(control)}$ (Smit et al., 2008), which evidenced a positive K-band and negative I-band, respectively. The insert presents the relative kinetics $\Delta W_{OK} = W_{OK(treatment)} - W_{OK(control)}$, $[W_{OK} = (F_t-F_o)/(F_K-F_o)]$ showed a positive L-band (Fig. 1*C*).

Table 1. Analysis of variance for photosynthetic parameters deduced by the JIP test in *B. porphyrostele* plants under infestation by mealybug. *F* values, significance levels (*p*) and the coefficient of variation (CV) are presented for each parameter.

Parameter	*F*	*p*	CV (%)	Significance
Tf(max)	7.737	0.031	22.96	*
Area	2.678	0.152	28.6	NS
Mo	16.841	0.006	14.08	**
F_o	0.139	0.721	34.72	NS
F_m	8.104	0.029	11.29	*
V_J	11.374	0.015	8.43	*
V_I	6.522	0.043	1.64	*
ABS/RC	12.656	0.012	13.79	*
ET_o/RC	0.081	0.785	4.83	NS
TR_o/RC	31.689	0.001	5.07	***
DI_o/RC	7.404	0.034	37.5	*
φ_{Po}	7.08	0.037	7.21	*
ψ_{Eo}	11.374	0.01	8.34	**
φ_{Eo}	12.025	0.013	13.37	*
φ_{Ro}	5.315	0.06	20.22	NS
δ_{Ro}	0.029	0.87	6.76	NS
ρ_{Ro}	6.108	0.048	12.1	*
φ_{Do}	7.08	0.037	20.25	NS
RE_o/Rco	0.615	0.997	6.38	NS
Sm	1.11	0.332	16.36	NS
N	11.303	0.015	13.22	*
$\gamma_{RC}/(1-\gamma_{RC})$	19.934	0.004	10.33	**
$\varphi_{Po}/(1-\varphi_{Po})$	10.175	0.018	19.59	*
$\psi_{Eo}/(1-\psi_{Eo})$	16.068	0.007	14.08	**
$\delta_{Ro}/(1-\delta_{Ro})$	0.025	0.879	10.06	NS
PI_{ABS}	23.548	0.002	28.31	**
PI_{TOTAL}	17.479	0.005	31.95	*

NS(not significant), $p > 0.05$
* Significant level, $p \leq 0.05$
** Significance level, $p \leq 0.01$
*** Significance level, $p \leq 0.001$

To distinguish the sequence of events from exciton trapping by PSII up to plastoquinone reduction [O-I phase, W_{OI} from 0 to 1, $W_{OI} = (F_t-F_o)/(F_I-F_o)$, according to Yusuf et al., 2010], from the PSI-driven electron transfer to the end electron acceptors on the PSI acceptor side, starting at PQH_2 (plastoquinol) [I–P phases, $W_{OI} \geq 1 = (F_t-F_I)/(F_I-F_0)$, according to Lin et al., 2009], the average W_{OI} kinetics was calculated and showed in Fig. 1*D*. To infested plants, the curve W_{OI} evidenced a positive behavior between O-I steps and negative between I-P steps (Fig. 1*D*).

The JIP-test data was plotted in a spider-plot (radarplot center = 0.0, maximum = 2.5), and the values of non-infested plants were used as control reference values (Fig. 2). Plants presented visible symptoms when infested by *D. boisduvalii*, including slightly smaller and yellowing leaves, chlorotic patches and deformed leaves. However, the differences between the two sets of plants were more pronounced in terms of their photosynthetic performance. In this study, the relative differences between the infested and non-infested plants are presented in Fig 2, following the statistical analyze described in Table 1. *The technical parameters* of leaves infested by mealybug had increased Tf(max), dV/dt_o, V_J, V_I and *N*, but decreased Fm (Table 1, Fig. 2) while there were no change in Area, F_o and S_m. *The specific fluxes* ABS/RC, TR_o/RC and DI_o/RC also increased in response to mealybug infestation, without any significant change in ET_o/RC. To better evaluate damages on electron transport reactions between photosystems, the *yields or energy flux ratios* and the *vitality indexes* were analyzed in both infested and non-infested plants. The infestation by mealybug reduced φ_{Po}, ψ_{Eo}, φ_{Eo}, ρ_{Ro} and all vitality indexes of dark-adapted leaves, except $\delta_{Ro}/(1-\delta_{Ro})$ (Table 1, Fig. 2).

A large scale survey of orchids plant cultivated in São Mateus (Brazil) are infected with any type of virus, fungi or any insects specie. The attack of insect can be a serious problem in the floriculture industry, because it reduces the production of high-quality orchid plants for international market and domestic growers, giving great importance to the early detection of symptoms caused by mealybug infestations. In this study, the OJIP transients showed a typical polyphasic with the fluorescence signal rising from the initial fluorescence level (F_o) to the maximal level (F_m) with well-defined intermediate J and I steps. However, OJIP transient of infested leaves showed a rise at the O-step and a large depression at the P-step (Fig. 1*A*). All these results show that all samples were photosynthetically active (Yusuf et al., 2010), but also give us evidences of the higher heterogeneity of samples. The chlorophyll fluorescence measurements provided three distinct phases: O-J, J-I and I-P. According to Schansker et al. (2005), these phases reflect different reduction processes of the electron transport chain. The O-J phase reflects the reduction of the acceptor side of PSII. The phase J-I represent the kinetic properties for reduction/oxidation of the plastoquinone pool while the phase I-P reflects the re-reduction of plastocyanin and $P700^+$ in PSI (Schansker et al., 2005; Tóth et al., 2007).

A strongly relationship between the mealybug infestation and several Chl *a* fluorescence JIP-test parameters also was observed, indicating the use of this JIP-test parameters in studies diagnosing biotic stress caused by mealybug in orchid plants. Significant differences in the slope-related parameters, in the JIP-test parameters directly obtained from the recorded fluorescence transients and in the area-related parameters were found. To our knowledge, this is the first report on detection of foliar symptoms in *Brasiliorchis* plants under mealybug infection, using a non-destructive chlorophyll fluorescence method.

The area above the fluorescence curve between F_0 and F_M reduced dramatically in the infested plants (Fig 1*A*). The area represents the electron acceptor pool size of PSII which includes Q_A and Q_B pools (Joliot & Joliot, 2002). Reductions of area occur when the electron transport from reaction center to quinone pool is impaired or completely blocked (Mehta et al., 2010). In this study, the area over the fluorescence curve was decreased by 29% under mealybug infestation as compared to control leaves, showing that mealybug infestation inhibits the electron transfer rates at the donor side of PSII and decreases the pool size of Q_A^-. However, the levels of F_o were not affected significantly under pathogen-infestation but F_m decreased under mealybug infestation (Fig. 2). F_o is associated with the donator side of PSII, with the adjustment

capacity of the antenna pigment level or with the excitation trapping efficiency at the active center of PSII. Any damage level to the PSII reaction center is one of the most direct signs of inhibition of photosynthesis or photoinhibition (Calatayud et al., 2001; Jiao et al., 2003). Reductions of F_m value in infested plants reflect the decreasing of ability of PSII to reduce the primary quinone (Q_A). Therefore, they would diminish of the electron donation capacity of the PSII reaction centers that share a PQ-pool and slowdown the reduction of the PQ-pool and at the same time lead to a lowering of the F_m-level (Schansker et al., 2005; Schansker et al., 2006).

In this study we also observed that under conditions where a slight decrease of the ability of PSII to reduce the Q_A during the mealybug infestation, it was accompanied by a decline in the fraction of closed RCs at J step expressed such as proportion of the total number of the RCs that can be closed (Force et al., 2003), V_J [= $(F_J-F_o)/(F_m-F_o)$], evidencing the lost of Q_A re-oxidation capacity of orchids plants submitted to mealybug infestation. In this study, the positive amplitude of ΔV_t around 2ms (J-step) was used to indicate a higher biochemical inhibition of redox state of Q_A (Christen et al., 2007). The relative position of J step (V_J) associated to initial inclination M_0 give us also insights about the equilibrium between the excitation rate of PSII antennae and the transference rate of electrons behind Q_A^-. In this sense, the significant increase of V_J and M_o ($p \leq 0.05$) is an indicative of lower electron transport in infested plants probably due the higher activity of non-Q_A-reducing center. Q_A reducing centers are active centers while Q_A non-reducing centers are inactive centers (Mehta et al., 2010).

Furthermore, increases in V_I under mealybug infestation should be caused by an increase in F_I or a decrease in F_m. It is indicate a relative change in the proportion of Q_B-non-reducing PSII RCs, rather than an increase in absolute amount of the Q_B-non-reducing PSII RCs, which is one characteristic of photoinhibition of acceptor side (Setlik et al., 1990). Non-reducing PSII RCs act as efficient exciton traps but dissipate all their excitation energy as heat (Strasser et al., 2004). It is know that inactive PSII RCs can prevent further damage to themselves and protect neighboring active PSII RCs by acting as sinks of excessive energy.

A slight decrease, but significant, in the maximum yield of primary photochemistry of PSII ($\varphi_{Po} = F_v/F_m$) of infested plants was observed. Reductions in φ_{Po} should be better explained through of conversion of Q_A-reducing PSII RCs to Q_A-non-reducing PSII RCs or heat sinks (Hermans et al, 2003). According to Baker & Rosenqvist (2004), φ_{Po} is considered an indicator of photoinhinbitory damages to PSII complexes, changing the OLKJIP transients, which may be caused by both a decrease in F_m or an increase of F_o (Lin et al., 2009). However, photoinhibition is considered to be more accurately visualized trough of an increase in DI_o/RC and decrease in ψ_{Eo} than by a decline in φ_{Po} (Force et al., 2003; Jiang et al., 2008). The insensibility of φ_{Po} was also observed by Oukarroum et al. (2007) who studied drought and re-watering effects on the photosynthetic system in barley cultivars (*Hordeum vulgare* L.) by Chl *a* fluorescence OLKJIP.

DI_o/RC indicates the rate of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs (Force et al., 2003). Dissipation in this context refers to the loss of absorbed energy through heat, fluorescence and energy transfer to other systems (Strasser et al., 2000). Although the reduction of φ_{Po} has been considered a insensitive indicator of photoinhibition, our results support the occurrence of this phenomenon in *B. porphyrostele* orchid plants infested by mealybug. Photoinhibited plants show a lower photosynthetic capacity CO_2 fixation, which results in increases in both NADPH and ATP concentrations (Lin & Hsu, 2004).

With mealybug infestation, the ABS/RC values increased ($p \leq 0.05$). Furthermore, the increase in ABS/RC was consistent with the decrease of φ_{Po} and $\gamma_{RC}/(1-\gamma_{RC})$ (Fig. 2). ABS/RC i.e. effective antenna size of an active reaction centers, is calculated as a total number of photons absorbed by chlorophyll molecules of all RCs divided by total number of active RCs (Mehta et al., 2010). In this context, ABS/RC values are influenced by ratio of active/inactive RCs. Thus, the parameter ABS/RC can represent (i) inactive RCs transformed in Q_A-non-reducing PSII RCs,

when decreases in ABS/RC are observed in association with the stabilization of TR_0/RC and decreases in φ_{Po} and $\gamma_{RC}/(1-\gamma_{RC})$ or (ii) the functional size of antenna system resultant from proportional behavior between φ_{Po} and ABS/RC (Yusuf et al., 2010). This result indicates that the energy trapped was efficiently dissipated as heat under mealybug infestation. Thus, we can tough that was a conversion of Q_A-non-reducing PSII RCs due damages suffered on oxygen evolution complex (OEC) and, then, in heat dissipater. Higher DI_o/RC values and stabilized ET_o/RC values ($p > 0.001$) support this hypothesis.

As shown in Fig. 1*C*, infestation-induced stimulation of the K-band is observed at 300µs in *B. porphyrostele* plants. Positive values of K-band indicate damages within PSII between the acceptor and donator side, resulting in an imbalance between the electron flow from the OEC to the reaction center and towards the acceptor side of PSII in the direction of PSI (Strasser, 1997). This means that the infestation by mealybug caused damages on OEC inhibiting of electron donation from water to the secondary electron donor of PSII (Y_Z) (Srivastava et al., 1997; Strasser, 1997). Mechanisms have been reported in the literature to explain differences in the OEC stability between different plants species and stress conditions. The osmolyte glycine betaine has been shown to protect the OEC under conditions of drought and salinity (Papageorgiou & Murata, 1995). De Ronde et al. (2004) wrote that the uncoupling of the OEC enables an alternative internal electron donor instead of H_2O (such as proline or ascorbate) to donate electrons to PSII leading to a shortlived increase in the reduced Pheo/Q_A concentration, resulting in a K peak which increases with stress intensity and duration. The appearance of a K-band occurs also under heat stress conditions (Bukhov et al., 2001; De Ronde et al., 2004).

The shape of the induction curve between 0.05 and 0.3ms, which indicates that the excitation energy transfer between PSII units, commonly referred as connectivity or grouping (Strasser & Stirbet, 1998), was influenced by biotic stress caused by mealybug infestation. In this study, we observed positive amplitude for the L-band of orchid leaves indicating that the initial fluorescence rise was highly hyperbolic after infestation by mealybug (details in insert of Fig. 1*C*), reflecting in decrease of the energetic connection or grouping. On the physiological basis, grouping is referred to be sensitive to (de-) stacking of the thylakoid membranes (Oukarroum et al., 2007). De-stacking has been shown to occur in response to several stress conditions such as drought (Oukarroum et al., 2007, 2009) and phosphorus deficiency (Lin et al., 2009). Studies relating the influence of biotic stress on energetic connectivity between PSII units are scarce. The fluorescence rise during the first 2ms is related to primary photochemistry. Oukarroum et al. (2007) suggest that stimulated L and K-bands constitute potential indicator for physiological disturbances before appearance of visible signs of stress. Thus, the use of L and K-bands should be a useful tool in studies relating the stress in plants.

Our results also showed that mealybug infestation decreased all yields parameters (φ_{Po}, ψ_{Eo}, φ_{Eo}, φ_{Do}, δ_{Ro}, ρ_{Ro} and φ_{Ro}) and the reductional fluxes of the PSI end electron acceptors (RE_o/RC_o e RE_o/CS_o). This means that leaves infested by mealybug have damages of all photochemical e non-photochemical redox reactions, thus limiting the capacity for absorption, trapping, electrons transport and reductional events as evidenced by decreases in PI_{ABS} and PI_{total}, with higher damages on PSII compared to PSI, such as evidenced by component $\delta_{Ro}/(1-\delta_{Ro})$, which was unaffected (Figure 2 and Table 1). $\delta_{Ro}/(1-\delta_{Ro})$ refers to redox state of end acceptors at PSI electron acceptor side (Yusuf et al., 2010).

The performance index (also called of "vitality index" according to Christen et al., 2007) is one of the parameters used to analyze the responses of the plants to stress (Oukarroum et al., 2007). The vitality index is composed by redox reactions of photochemical phase O-J and the thermal phases J-I and I-P (Lazár, 1999) and according to Strasser et al. (2004), PI has been calculated from three (PI_{ABS}) or four (PI_{total}) components which depend: $\gamma_{RC}/(1-\gamma_{RC})$, an expression related to density of active PSII, an expression related to primary photochemistry $\varphi_{Po}/(1-\varphi_{Po})$, a component that describes the performance of the dark redox reactions of intersistem $\psi_{Eo}/(1-\psi_{Eo})$ and the end acceptors at PSI acceptor side $\delta_{Ro} / (1 - \delta_{Ro})$ (Strasser et al.,

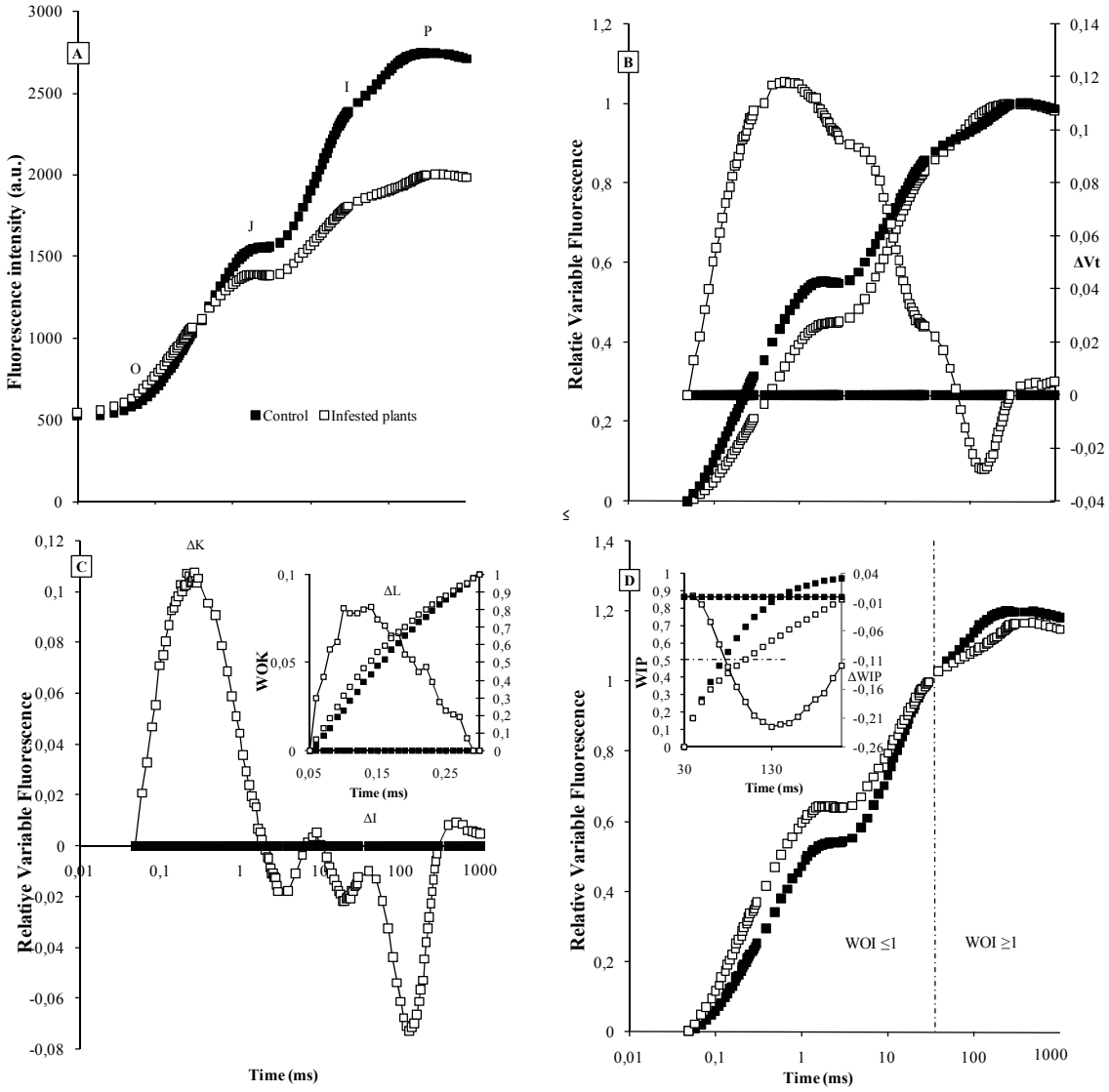

Figure. 1. Effect of mealybug infestation on the Chl *a* fluorescence in *B. porphyrostele* orchid plants based on the analysis of the OJIP fluorescence transients. [*A*]: average kinetics from fluorescence intensity, [*B*]: relative variable fluorescence (F_t) and their relative subtraction ΔV_t, [*C*]: relative variable fluorescence where $\Delta W_{OJ} = W_{OJ(treatment)} - W_{OJ(control)}$ between 0.05 and 2 ms and $\Delta W_{JP}=W_{JP}(treatment) - W_{JP}(control)$ between 2 and 300 ms, which describe K and I-band, respectively. In the insert: relative fluorescence $\Delta W_{OK} = W_{OK(treatment)} - W_{OK(control)}$, $[W_{OK} = (F_t-F_0)/(F_K-F_0)]$ or L-band, [*D*]: relative fluorescence variable $W_{OI} = (F_t-F_0)/(F_I-F_0)$. In the insert: relative fluorescence $\Delta W_{IP} = W_{IP(treatment)} - W_{IP(control)}$, $[W_{IP} = (F_t-F_I)/(F_P-F_I)]$. (■) Control and (□) Infested plants. (n = 5)

2004; Tsimilli-Michael & Strasser, 2008). PI values can be altered when an environmental stress affects any of these components, showing that PI values have a higher sensitivity than that achieved by any of its isolated components.

In this study, we also compared the sequence of events from exciton trapping by PSII (presented as O-I phase) from the PSI driven electron transfer to the end electron stress affects any of these components, 2004; Tsimilli-Michael & Strasser, 2008). PI values can be altered when an environmental showing acceptors on the PSI acceptor side (I-P phase) (Yusuf et al., 2010) between infested and non-infested plants (Fig. 1*D*). The higher amplitude of O-I phase observed on infested plants shows that the acceptor side of PSII was more reduced (left from vertical dash line on Fig. 1*D*), while the acceptor side of PSI was more oxidized (right from vertical dash line on Fig. 1*D*). This result demonstrates higher occurrence of damages on acceptor side of PSII under mealybug infestation. This result has been also shown to occur in response to abiotic stress conditions (Lin et al., 2009). Furthermore, infested plants showed reduced amplitude of I-P phase (W_{IP} - Fig. 1*D*) which suggests the occurrence of process leading to the reduction of the pool of the end electron acceptors at PSI acceptor side (Tsimilli-michael & Strasser, 2008; ($W_{IPtreatment}$–$W_{IPcontrol}$) which the Yusuf et al., 2010).This was further elucidated by evaluating the relative subtraction ΔW_{IP} revealed the negative amplitude around 130 ms (right axis in insert of Fig. 1*D*). Negative amplitude of ΔW_{IP} serves to estimate the K_m value for the reduction of ferredoxin-Fd, other intermediates and NADP (Yusuf et al., 2010). This achieve give us a strongly argument to accept the hypothesis which suggest higher damages on PSII photochemistry compared to PSI. Finally, when we related $W_{OI} \geq 1$ with W_{IP} (see details in Fig. 1*D*), some dependence between the overall rate constant for the reduction of the end electron acceptor pool and the regulation of the pool size was observed on infested leaves compared to control plants. This means that the decreased rate of reduction was proportional to reduction of the end electron acceptor pool. However, Yusuf et al. (2010) encountered independence between $W_{OI} \geq 1$ with W_{IP} in *Brassica juncea* plants under alleviates abiotic stress.

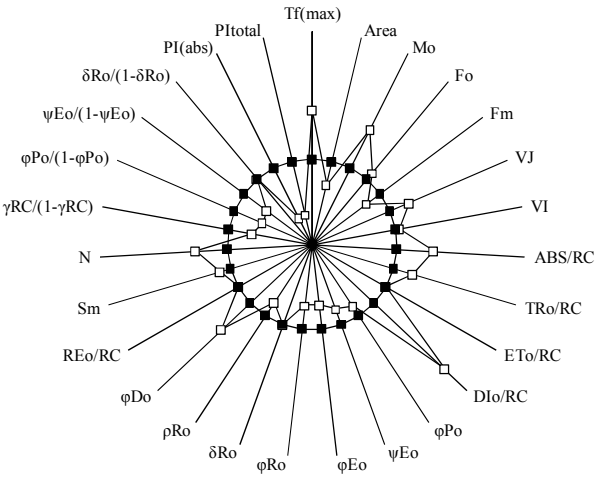

Figure. 2. Effect of mealybug infestation on functional and structural JIP-test parameter. The JIP-test data was plotted in a spider-plot (radarplot center = 0.0, maximum = 2.5), and the values of non-infested plants were used as control reference values. (■) Control and (□) Infested plants. The derivation of each parameter and the statistical analyse of the JIP-test are presented in Table 1 and Table 2, respectively. (n = 5)

The results of this study show that reduced photochemical capacities of orchid plant mealybug-affected could be due to an increased susceptibility to potoinhibition. This may possibly explain the cultural practice of farmers, which growing the *Brasiliorchis* plants in shade and increasing shading, when the plants show increased severity of infestation, which normally increases with the age of the plant. Increasing shading we reduce the risks of damages to photosynthetic apparatus. In these conditions, the plants could be able to synthesize more photosynthetic products, resulting in increased quality of flowers. Also, previous studies have been shown that pathogen-free orchid present higher inflorescence length as well as the number of side branches as compared to those infected orchid (Chia & He, 1999). It may not only be due to the higher photochemical capacities but also to the stronger sink strength of the inflorescence. Besides, on theorical basis, the main cause of reduction on photosynthetic capacity is believed to be due to stomatal limitations, which leads to a decrease of CO_2 assimilation and, consequently, of photosynthetic products synthesis.

CONCLUSION

In summary, the use of OLKJIP fluorescence transients measurements and their analysis through of JIP-test (e.g. performance index PI, change in the L and K-bands, specific energy fluxes) to assay biotic-stressed *Brasiliorchis* plants was very useful and informative. Our data shows that mealybug infestation decreased all yields parameters and the reductional fluxes of the PSI end electron acceptors, resulting in damages on all photochemical e non-photochemical redox reactions. In addition, decreases of the energetic connection or grouping and damages on OEC were observed on leaves infested by mealybug. Furthermore, negative amplitudes of ΔI and ΔW_{IP} suggest higher damages on PSII photochemistry compared to PSI. Thus, the chlorophyll fluorescence analyses used in this study provided clear indication of the alternations in photochemical activities of plants caused by mealybug attack.

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