PHOTOSYNTHETICA 35 (1): 29-39, 1998

Chlorophyll fluorescence parameters characterizing the development of two cacao genotypes infected by witches' broom

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Abstract

Greenhouse-grown susceptible 20-d-old seedlings of *Theobroma cacao* genotypes Catongo and tolerant genotype SCA6×Catongo were inoculated with a mixture of isolates of *Crinipellis perniciosa,* the causal agent of witches' broom. The characteristics of chlorophyll *a* fluorescence emission were monitored during leaf ontogeny using a portable system *PAM-2000.* In both inoculated and non-inoculated genotypes, significant differences were found for the effective quantum yield values of photosystem (PS) 2 ($\Delta F/F_m'$) at the B (7 to 14-d-old), D (21 to 30-d-old), and E (>30-d-old) stages of leaf development, and in quantum yield of the non-cyclic photosynthetic electron transport between PS2 and PS1 $[q_P(F_v/F_m)]$ and quencher efficiency $[(F_m-F_t)/F_0]$ at the B, C (15 to 20-d-old) and D stages. Intergenotypic differences were found only for the $[q_P(F_v/F_m)]$ and $[(F_m-F_t)/F_0]$ values at the E stage, and for fluorescence quenching (F_m-F_t) at the B and E stages. Highly significant inter- and intragenotype relationships were found between the rate of photosynthetic electron transport to PS2 (A_{max}) and maximum fluorescence during actinic irradiation (F_m'). Also, each of the highly significant relationships between (F_m-F_t) and A_{max}, $[(F_m-F_t)/F_0]$ and $\Delta F/F_m'$, and between $[(F_m-F_t)/F_0]$ and A_{max} were represented by a general model, independent of treatments. Therefore, alterations in energy distribution in the radiant energy collector complex interior of PS2 and reduction in absorption of photosynthetically active radiation were observed in the infected plants, mainly in the hybrid at the C stage. Also, variations were found in the non-cyclic photosynthetic electron transport at the B and C stages in the infected Catongo.

Additional key words: *Crinipellis perniciosa*; genotypes; hybrids; leaf development; pathogens; photosystems 1 and 2; quantum yield; *Theobroma cacao*.

Introduction

After the invasion of pathogens to a host tissue, an increase in photosynthetic activity

Received 5 November 1996, *accepted* 18 June 1997.

is observed for a short period, followed by a decrease due to destruction or degradation of photosynthetic pigments (Scholes and Farrar 1985, Leite and Paschoiati 1995). Pathogens act specifically, either by interrupting the photosynthetic electron transfer, or by diminishing the quantity of photosynthetic proteins (Scholes 1992). Goodman *et al.* (1986) affirm that some fungus can induce the decrease in non-cyclic photosynthetic electron transport and in oxidative photophosphorylation. This was demonstrated by the fall in number of electron carriers, mainly cytochromes, in infected leaves when compared to healthy tissue. In some cases, mesophyll cell chloroplasts of some plant species infected by *Puccinia* work in a form similar to healthy leaves, however, the photosynthetic rate is reduced due to chloroplast destruction (Ahmad *et al.* 1983).

Kinetic measurements of chlorophyll (Chl) *a* fluorescence induction monitor effects of several stresses over the photosynthetic apparatus. Furthermore, changes in the emission, during photosynthesis induction, are closely related to CO_2 assimilation rate (Ogawa 1982, Walker *et al.* 1983, Ireland *et al.* 1984) and to evolution of photosynthetic O_2 (Horton 1983, Walker *et al.* 1983, Bradbury and Baker 1984). Although the Chl fluorescence kinetic reveals complex changes in energy balance in the thylakoids interior, each phase of the fluorescence curve results from a particular biophysical/biochemical process governed by the energy flux through proteinaceous complexes in thylakoids.

Crinipellis perniciosa (Stahel) Singer, the causal agent of witches' broom of *Theobroma cacao*, presents two different morpho-physiological phases during its life cycle, a parasitic and a saprophytic. In the first phase the pathogen needs the vegetative and/or reproductive growing host tissue for its nutrition, and in the saprophytic phase it nourishes from dead tissue. Therefore, the objective of this work was to study and evaluate possible damages caused by *C. perniciosa* during leaf ontogeny of *T. cacao*, through measurements of the kinetics of Chl fluorescence in the slow phase.

Materials and methods

Genetic material and crop conditions: Seeds of tolerant, SCA 6×Catongo, and susceptible, Catongo, genotypes to witches' broom disease germinated in humid sawdust for 5 d. Plants were transferred to polyethylene bags of 2 kg capacity filled with *Alfisol* (*Tropudalf*). The seedlings were maintained in a greenhouse which permitted solar radiation reduction of 30 % of the maximum irradiance (2500 μmol m^{-2} s^{-1}).

Inoculation system: The seedlings were inoculated using methods described by Frias (1987), 20 d after emergence utilizing a 2×10^5 basidiospores per cm^3 mixture of isolates of *C. perniciosa*. After inoculation the seedlings were maintained in a chamber at 25±2 °C and 100 % relative humidity for 24 h. At the end of incubation period, the seedlings were transferred to a greenhouse, and irrigated periodically with tap water and concentrated nutrient solution (Hoagland and Arnon 1938).

Chl fluorescence measurements were done at the B, C, D, and E stages of leaf development, corresponding to 7-14, 15-20, 21-30, and >30 d of leaf age, respectively (Merkel *et al.* 1994). Leaves at stage A (0-6 d) were not used due to their small size. The fluorescence measurements were done using a portable system (*PAM-2000, H. Walz,* Effeltrich, Germany) [actinic source: peak 655 nm, 600 μmol(photon) m^{-2} s^{-1}; saturating pulse: λ< 710 nm, 15 000 μmol m^{-2} s^{-1}]. With the actinic radiation, photosynthetic activity and the concomitant changes of Chl fluorescence are induced. In order to determine the maximum fluorescence before (F_0) and during steady state (F_m'), and photochemical (q_P) and non-photochemical (q_{NP}) quenching, a saturating pulse was given before the induction and at the steady state of induction of Chl fluorescence. The measurements began 12 d after inoculation. At each measurement, a clip was fixed to the leaf prior to the irradiation. By the end of this dark adaptation, all the reaction centres were "open" (unreduced quencher). For the detection of the fluorescence signal, a sensor of optical fiber was coupled to the clip. The fluorescence signals were registered in a data acquisition system *DA-2000* which automatically calculated maximum (F_m) and terminal (F_t) fluorescences, F_m', effective quantum yield of PS2 ($\Delta F/F_m'$), q_P, and q_{NP}. From these measurements we determined: the fluorescence decrease ratio $[(F_m-F_t)/F_t]$, fluorescence quenching (F_m-F_t) (Jenkins *et al.* 1981), quencher efficiency $[(F_m-F_t)/F_0]$ (Ahmad *et al.* 1983), quantum yield of the non-cyclic photosynthetic electron transport between PS2 and PS1 $[q_P(F_v/F_m)]$ (Genty *et al.* 1989), and the rate of photosynthetic electron transport to PS2 (A_{max}) which corresponds to the area above the curve between F_0 and F_m (Bolhàr-Nordenkampf and Öquist 1993).

Experimental design: The seedlings were arranged in a complete randomized design with four replications, and 10 plants per experimental unit. Analyses of variance were done using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute 1987). Mean ranking was done using the Duncan Multiple Range test at 5 % probability. Regression and correlation analyses of the parameters were also performed. Homogeneity of regression coefficients was tested according to Steel and Torrie (1980).

Results and discussion

Inter- and intragenotypic variations were observed through leaf ontogeny for the parameters of the kinetics of Chl fluorescence in the slow phase. The F_m values, determined before actinic irradiation, differed inter- and intragenotypically at the B, C, and E stages (Table 1). At the C stage, a decrease in F_m values was found for both infected genotypes, mainly for the hybrid. This was also verified at the E stage for the infected hybrid (Table 1). Similar results were detected in *Phaseolus vulgaris* leaves, 7 d after inoculation with *Uromyces appendiculatus* (Peterson and Aylor 1995). Intergenotypic differences were observed also for F_m' at the C stage of leaf development: Catongo showed the lower value (Table 1).

Table 1. Maximum (F_m) fluorescence, maximum fluorescence during actinic irradiation (F_m'), terminal fluorescence (F_t), and effective quantum yield of PS2 ($\Delta F/F_m'$) measurements at the B, C, D, and E stages of leaf development of two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. At each stage and parameter, means followed by the same letter in the same column did not differ according to the Duncan's Multiple Range Test ($p \leq 0.05$). Mean values of 10 measurements (±s). CI: Catongo infected, CC: Catongo control, HI: Hybrid infected, HC: Hybrid control. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

Parameter	Treat.	Stage of leaf development			
		B	C	D	E
F_m	CI	0.20(±0.010)ab	0.20(±0.010)bc	0.37(±0.024)a	0.37(±0.010)c
	CC	0.18(±0.011)b	0.23(±0.022)b	0.38(±0.015)a	0.37(±0.006)c
	HI	0.23(±0.008)a	0.18(±0.009)c	0.35(±0.011)a	0.40(±0.007)b
	HC	0.22(±0.009)a	0.30(±0.018)a	0.36(±0.013)a	0.43(±0.007)a
F_m'	CI	0.15(±0.006)a	0.15(±0.014)b	0.29(±0.023)a	0.30(±0.009)a
	CC	0.15(±0.012)a	0.19(±0.020)ab	0.28(±0.010)a	0.30(±0.006)a
	HI	0.17(±0.011)a	0.18(±0.014)ab	0.25(±0.011)a	0.28(±0.007)a
	HC	0.14(±0.009)a	0.20(±0.013)a	0.28(±0.016)a	0.30(±0.010)a
F_t	CI	0.11(±0.006)a	0.10(±0.008)b	0.15(±0.008)ab	0.16(±0.007)a
	CC	0.10(±0.007)a	0.13(±0.008)a	0.16(±0.006)a	0.16(±0.007)a
	HI	0.11(±0.007)a	0.12(±0.007)ab	0.13(±0.005)b	0.14(±0.003)b
	HC	0.10(±0.005)a	0.12(±0.006)ab	0.14(±0.008)b	0.15(±0.005)ab
$\Delta F/F_m'$	CI	0.27(±0.019)b	0.28(±0.035)a	0.45(±0.030)ab	0.46(±0.019)b
	CC	0.32(±0.011)a	0.29(±0.045)a	0.41(±0.018)b	0.46(±0.022)b
	HI	0.31(±0.012)ab	0.32(±0.035)a	0.46(±0.024)ab	0.52(±0.009)a
	HC	0.32(±0.012)a	0.37(±0.020)a	0.51(±0.019)a	0.51(±0.009)ab

The quenching of Chl fluorescence from F_m to F_t depends on oxido-reduction reactions of the primary electron acceptor (Q_A) and on the plastoquinone (PQ) pool size. It is also related to the build up of an electrochemical gradient ($\Delta\psi$) across the thylakoid membranes (Scholes and Farrar 1985). The drop in F_m values can be attributed to alterations of energy distribution in the interior of the light-harvesting complex, and to reduction of absorption of photosynthetically active radiation by chloroplast pigments (Stuhlfauth *et al.* 1988). The fall of F_m' implies an increase of non-photochemical quenching (Raggi 1995). The decrease of F_m' values results in formation of a pH gradient (ΔpH), followed by a decrease in the quantum yield of PS2 (Genty *et al.* 1989). The invariability in the F_0 and F_m values implies the existence of a perfect energy distribution in the interior of the light-harvesting complex of PS2 (Stuhlfauth *et al.* 1988). Furthermore, it indicates that the Chl molecules of PS2 are intact, and their capacity for absorption of quanta remains constant (Stuhlfauth *et al.* 1988). The F_m values are proportional to the quantity of Chl *a* molecules in leaf tissue (Miranda *et al.* 1981). The increase in F_m may also be attributed to phosphorylation of proteins in thylakoid membranes (Horton and Black 1983).

Inter- and intragenotypically, the values of effective quantum yield of PS2 ($\Delta F\ F_m'$) differed at the B, D and E stages, and those of F_t at the C, D and E stages (Table 1). Furthermore, highly significant linear and positive relationships between (F_m-F_t) and A_{max}, explained by a single model (Fig. 1), and between A_{max} and F_m' (Fig. 2), were found. Differences in regression coefficients of the latter relationship were also detected using the test of homogeneity of slopes (Steel and Torrie 1980). The relationship showed by infected genotypes was significantly different from that of healthy seedlings (Fig. 2).

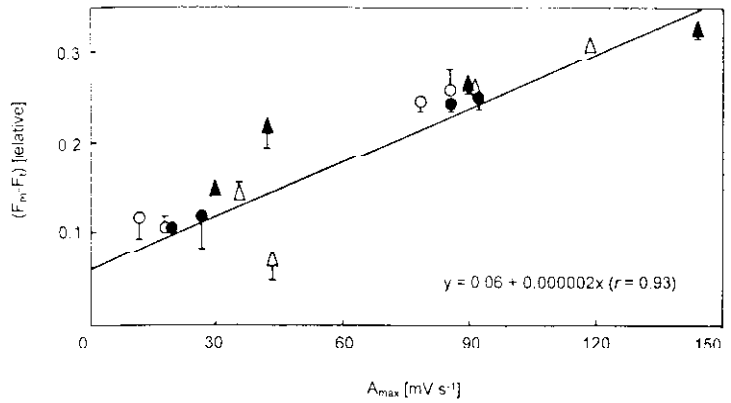

Fig. 1. Relationship between fluorescence quenching (F_m-F_t) and photosynthetic electron transport to PS2 (A_{max}) in two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. Catongo infected O, Catongo control ●, Hybrid infected Δ, Hybrid control ▲. Each measurement is the mean (±s) value of 10 replications. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

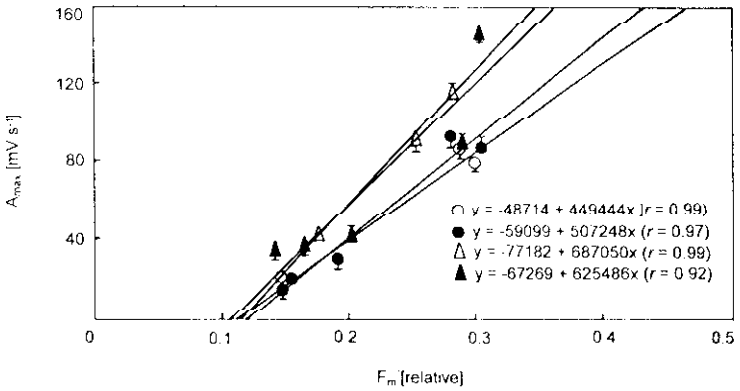

Fig. 2. Relationship between photosynthetic electron transport to PS2 (A_{max}) and maximum fluorescence during actinic irradiation (F_m') in two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. Catongo infected O, Catongo control ●, Hybrid infected Δ, Hybrid control ▲. Each measurement is the mean (±s) value of 10 replications. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

The F_t yield is associated with the equilibrium between the reduction of Q_A, *via* PS2, and the oxidation of Q_A through PS1 (Krause 1973). The quantum efficiency of PS2 can be determined through F_t and F_m' (Genty *et al.* 1989). The A_{max} is proportional to the pool size of electron acceptors on the reducing site of PS2 (Bolhàr-Nordenkampf and Öquist 1993). With the reduction in PQ pool, the probability of excitation energy utilization by PS2 decreases, and the dissipation of Chl energy by other photochemical processes increases, rising the emission of Chl fluorescence (Baker and Bradbury 1981). A moderate decrease in $\Delta F/F_m'$ and the steep fall in the net CO_2 fixation are important characteristics of infected tissue (Raggi 1995). Furthermore, the inhibition of CO_2 fixation, and the decline of variable to maximum fluorescence ratio and of $\Delta F/F_m'$ values indicate changes in the flux of energy (Raggi 1995). Therefore, changes in quantum yield of the non-cyclic photosynthetic electron transport can be estimated through the determination of $\Delta F/F_m'$ (Genty *et al.* 1989).

Table 2. Fluorescence quenching (F_m-F_t), fluorescence decrease ratio [(F_m-F_t)/F_t], and quencher efficiency [(F_m-F_t)/F_0] measurements at the B, C, D, and E stages of leaf development of two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. At each stage and parameter, means followed by the same letter in the same column did not differ according to Duncan's Multiple Range Test ($p \leq 0.05$). Mean (±s) values of 10 measurements. CI: Catongo infected, CC: Catongo control, HI: Hybrid infected, HC: Hybrid control. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

Parameter	Treat.	Stage of leaf development			
		B	C	D	E
(F_m-F_t)	CI	0.09(±0.010)b	0.10(±0.017)b	0.22(±0.019)a	0.21(±0.010)b
	CC	0.08(±0.008)b	0.10(±0.028)b	0.22(±0.018)a	0.21(±0.012)b
	HI	0.12(±0.009)a	0.06(±0.016)c	0.22(±0.014)a	0.26(±0.006)a
	HC	0.12(±0.006)a	0.18(±0.017)a	0.22(±0.007)a	0.28(±0.006)a
[(F_m-F_t)/F_t]	CI	0.82(±0.130)b	1.00(±0.580)b	1.47(±0.102)ab	1.31(±0.088)b
	CC	0.80(±0.106)b	0.77(±0.320)bc	1.38(±0.146)b	1.31(±0.120)b
	HI	1.09(±0.133)a	0.50(±0.203)c	1.69(±0.160)a	1.86(±0.052)a
	HC	1.20(±0.049)a	1.50(±0.239)a	1.57(±0.045)ab	1.87(±0.071)a
[(F_m-F_t)/F_0]	CI	0.82(±0.030)b	0.91(±0.080)ab	1.69(±0.072)a	1.40(±0.098)b
	CC	0.89(±0.006)ab	0.91(±0.020)ab	1.57(±0.046)b	1.62(±0.070)b
	HI	1.20(±0.043)a	0.50(±0.093)b	1.69(±0.060)a	2.36(±0.052)a
	HC	1.20(±0.049)a	1.29(±0.039)a	1.83(±0.045)a	2.80(±0.060)a

Inter- and intragenotypic differences were found in fluorescence decrease ratio [(F_m-F_t)/F_t] at the C and D stages, and in quencher efficiency [(F_m-F_t)/F_0] at the B, C, and D stages (Table 2). Differences in (F_m-F_t), were shown only at the C stage (Table 2). Nevertheless, intergenotypic differences in (F_m-F_t) and [(F_m-F_t)/F_t] were found at the B and E stages (Table 2). In addition, linear and highly significant relationships between [(F_m-F_t)/F_0] and $\Delta F/F_m'$ (Fig. 3) and between [(F_m-F_t)/F_0] and

A_{max} (Fig. 4) were detected. There were no statistical differences among the regression coefficients in these particular relationships (Figs. 3 and 4).

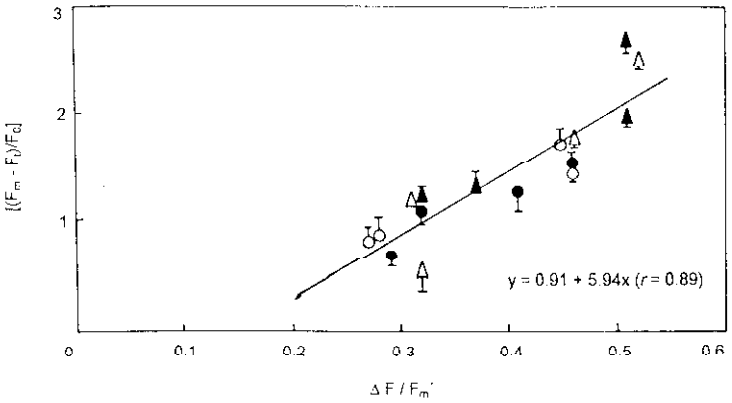

Fig. 3. Relation between quencher efficiency $[(F_m-F_t)/F_0]$ and effective quantum yield of PS2 $(\Delta F/F_m')$ in two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. Catongo Infected O, Catongo control ●, Hybrid infected Δ, Hybrid control ▲. Each measurement is the mean (±s) value of 10 replications. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

The stability in $[(F_m-F_t)/F_t]$ and in the time needed to reach half the difference between F_m and F_t values are related to the stability of the photochemical quenching, implying a stable flux of electrons through PS2 (Epron and Dreyer 1990). Nevertheless, the recovery of $[(F_m-F_t)/F_t]$ after F_m suggest the reacquisition of the oxidative properties of Q_A and enhancement in the energizing of the thylakoid system, the consequence of rebuilding a secondary gradient of metallic cations (Krause 1973, Krause *et al.* 1982). Ahmad *et al.* (1983) observed that mean values of $[(F_m-F_t)/F_0]$ were constant in *Hordeum vulgare* leaves infected by *Puccinia hordei,* and decreased in control leaves. In healthy leaves of this species, the activity of PS2, the capacity of generating an electrochemical gradient in the thylakoid membranes, and ATP production decreased with leaf ontogeny, but there was no inhibition of the photochemical processes of photosynthesis in infected leaves.

Values of $[(F_m-F_t)/F_0]$ can be used effectively as *in vivo* indicators of photosynthetic capacity, since they are directly related to the photosynthetic electron transport resulting from the electrical potential and the ionic concentration gradient around the thylakoid membranes (Miranda *et al.* 1981).

Inter- and intragenotypic differences of q_P values were found at the B, C, and E stages and for quantum yield of the non-cyclic photosynthetic electron transport between PS2 and PS1 $[q_P(F_v/F_m)]$ at the B, C, and D stages (Table 3). Intergenotypic differences of q_{NP} and $[q_P(F_v/F_m)]$ were found only at the E stage (Table 3).

Variations in q_P can be related to non-cyclic photosynthetic electron transport (Krause *et al.* 1982). These variations are attributed to differences between the absorbed radiant energy and the photosynthetic electron transport rate (Demmig and

Winter 1988). In light, a linear relationship can exist between q_P and $[q_P(F_v/F_m)]$ due to limitations of the non-cyclic photosynthetic electron transport rate at the reaction centres of PS2 (Genty *et al.* 1989). Nevertheless, Harbinson *et al.* (1989) verify that the quenching processes do not show consistent correlation with the quantum efficiency of the non-cyclic photosynthetic electron transport. However, an increase in the deactivation of excitons, through formation and liberation of heat, originates a type of q_P dependent on energy (Bolhàr-Nordenkampf and Öquist 1993).

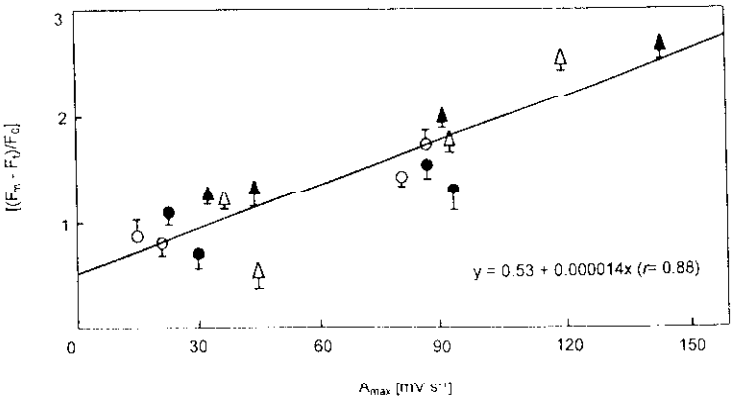

Fig. 4. Relation between quencher efficiency $[(F_m-F_t)/F_0]$ and photosynthetic electron transport rate to PS2 (A_{max}) in two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. Catongo infected O, Catongo control ●, Hybrid infected Δ, Hybrid control ▲. Each measurement is the mean (±s) value of 10 replications. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

A mechanism at chloroplast level, responsible for protection of the photosynthetic apparatus against environmental stresses, seems to be regulated by the thermal dissipation of excitation energy of PS2, resulting from the ΔpH-dependent increase in q_{NP} (Krause and Behrend 1986). Thus, q_{NP} originates almost entirely by thermal deexcitation of PS2, associated with rebuilding of a transthylakoidal proton gradient (Krause *et al.* 1988). In leaves of *Hyacinthoides non-scripta* inoculated with *Uromyces muscari*, fluorescence quenching in the pustules before sporulation was reduced to one half, suggesting a general loss of integrity of thylakoid membranes during the disease progression (Scholes and Farrar 1985). The regulation of PS2 by q_{NP} can be done by decreasing the energy of excitation that reaches the reaction centres of PS2 (Weis and Berry 1987). The ΔpH-dependent quenching, caused by the increase in thermal deactivation of the excitation states, originates from structural alterations in the membranes due to the exchange of H^+/Mg^{2+} at the internal thylakoid surface (Krause *et al.* 1983). Research in *Pisum sativum*, grown at 23 °C, showed that the alterations of q_{NP} values imply variations in Chl fluorescence induction (Bradbury *et al.* 1985). Therefore, the increase in q_{NP} values is related to the drop of thermal deexcitation, offset by the decline in photosynthetic electron transport (Epron and Dreyer 1992).

In conclusion, inter- and intragenotypic variations were found in the Chl *a* fluorescence parameters during the leaf developmental stages of the two genotypes studied. Alterations in energy distribution in the interior of the radiant energy collector complex of PS2, and reduction in absorption of photosynthetic active radiation were observed in the infected plants, mainly in the hybrid at the C stage. Also, variations were found in the non-cyclic photosynthetic electron transport, mainly at the B and C stages in the infected Catongo.

Table 3. Photochemical (q_P) and non-photochemical (q_{NP}) quenching and quantum yield of the non-cyclic photosynthetic electron transport between PS2 and PS1 [$q_P(F_v/F_m)$] measurements at the B, C, D, and E stages of leaf development of two genotypes of *Theobroma cacao* infected and non-infected with *Crinipellis perniciosa*. At each stage and parameter, means followed by the same letter in the same column did not differ according to Duncan's Multiple Range Test ($p \leq 0.05$). Mean (±s) values of 10 measurements. CI: Catongo infected, CC: Catongo control, HI: Hybrid infected, HC: Hybrid control. Susceptible Catongo and tolerant hybrid genotypes to *C. perniciosa*.

Parameter	Treat.	Stage of leaf development B	C	D	E
q_P	CI	0.85(±0.043)b	0.89(±0.055)a	0.93(±0.026)a	0.90(±0.023)a
	CC	0.91(±0.024)ab	0.71(±0.092)b	0.86(±0.031)a	0.82(±0.032)b
	HI	0.93(±0.026)ab	0.90(±0.041)a	0.90(±0.039)a	0.87(±0.012)ab
	HC	0.95(±0.019)a	0.96(±0.017)a	0.91(±0.019)a	0.83(±0.012)b
q_{NP}	CI	0.54(±0.064)a	0.42(±0.097)a	0.34(±0.054)a	0.34(±0.022)b
	CC	0.59(±0.027)a	0.32(±0.089)a	0.44(±0.050)a	0.31(±0.019)b
	HI	0.59(±0.042)a	0.43(±0.087)a	0.40(±0.063)a	0.41(±0.020)a
	HC	0.63(±0.028)a	0.51(±0.093)a	0.42(±0.054)a	0.41(±0.021)a
$[q_P(F_v/F_m)]$	CI	0.38(±0.034)b	0.40(±0.051)b	0.60(±0.031)a	0.53(±0.020)b
	CC	0.46(±0.020)ab	0.37(±0.063)b	0.54(±0.027)b	0.53(±0.024)b
	HI	0.53(±0.023)a	0.30(±0.056)c	0.57(±0.031)ab	0.64(±0.007)a
	HC	0.52(±0.013)a	0.51(±0.030)a	0.61(±0.012)a	0.64(±0.009)a

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