

# **non-photochemical excitation quenching Alteration of photosystem II properties with**

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**PHILOSOPHICAL**<br>TRANSACTIONS ৳ doi: 10.1098/rstb.2000.0702 Phil. Trans. R. Soc. Lond. B 2000 **355**, 1405-1418

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# THE ROYAL<br>
SOCIETY<br> **Alteration of photosystem II properties with non-photochemical excitation quenching**

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artment of Plant Physiology, Institute of Molecular and Cell Biology, University of Tartu, Riia Street 23, Tartu 51010, Estoni<br>Oxygen yield from single turnover flashes and multiple turnover pulses was measured in sunflowe Oxygen yield from single turnover flashes and multiple turnover pulses was measured in sunflower leaves differently pre-illuminated to induce either 'energy-dependent type' non-photochemical excitation quenching  $(a_1)$  or Oxygen yield from single turnover flashes and multiple turnover pulses was measured in sunflower leaves<br>differently pre-illuminated to induce either 'energy-dependent type' non-photochemical excitation<br>quenching ( $q_E$ ) or differently pre-illuminated to induce either 'energy-dependent type' non-photochemical excitation<br>quenching ( $q_E$ ) or reversible, inhibitory type non-photochemical quenching ( $q_I$ ). A zirconium O<sub>2</sub><br>analyser, combined wit quenching  $(q_E)$  or reversible, inhibitory type non-photochemical quenching<br>analyser, combined with a flexible gas system, was used for these measurem<br>saturating single turnover flashes was the equivalent of 1.3–2.0 µmol e  $-2$  in ing  $(q_1)$ . A zirconium  $O_2$ <br>ements. Oxygen yield from<br>in leaves pre-adapted to low<br>ure to saturating light, but it analyser, combined with a flexible gas system, was used for these measurements. Oxygen yield from<br>saturating single turnover flashes was the equivalent of  $1.3-2.0 \mu$  mole<sup>-</sup> m<sup>-2</sup> in leaves pre-adapted to low<br>light. It d saturating single turnover flashes was the equivalent of  $1.3-2.0\,\mu$ mol e<sup>-</sup> m<sup>-2</sup> in leaves pre-adapted to low<br>light. It did not decrease when  $q_E$  quenching was induced by a 1 min exposure to saturating light, but it<br>d light. It did not decrease when  $q_E$  quenching was induced by a 1 min exposure to saturating light, but it decreased when pre-illumination was extended to 30–60 min. Oxygen evolution from saturating multiple turnover puls decreased when pre-illumination was extended to 30–60 min. Oxygen evolution from saturating multiple<br>turnover pulses behaved similarly: it did not decrease with the rapidly induced  $q_E$  but decreased<br>considerably when exp turnover pulses behaved similarly: it did not decrease with the rapidly induced  $q_E$  but decreased considerably when exposure to saturating light was extended or  $O_2$  concentration was decreased to 0.4%.<br>Parallel recordi considerably when exposure to saturating light was extended or  $O_2$  concentration was decreased to 0.4%.<br>Parallel recording of chlorophyll fluorescence and  $O_2$  evolution during multiple turnover pulses,<br>interpreted wit Parallel recording of chlorophyll fluorescence and  $O_2$  evolution during multiple turnover pulses,<br>interpreted with the help of a mathematical model of photosystem II (PS II) electron transport, revealed<br>PS II donor and interpreted with the help of a mathematical model of photosystem II (PS II) electron transport, revealed<br>PS II donor and acceptor side resistances. These experiments showed that PS II properties depend on the<br>type of non-PS II donor and acceptor side resistances. These experiments showed that PS II properties depend on the type of non-photochemical quenching present. The rapidly induced and rapidly reversible  $q_E$  type (photoprotective) q type of non-photochemical quenching present. The rapidly induced and rapidly reversible  $q_E$  type (photoprotective) quenching does not induce changes in the number of active PS II or in the PS II maximum turnover rate, th (photoprotective) quenching does not induce changes in the number of active PS II or in the PS II maximum turnover rate, thus confirming the antenna mechanism of  $q_E$ . The more slowly induced but still reversible  $q_I$  typ maximum turnover rate, thus confirming the antenna mechanism of  $q_E$ . The more slowly induced but still reversible  $q_I$  type quenching (photoinactivation) induced a decrease in the number of active PS II and in the maximu reversible  $q_1$  type quenching (photoinactivat<br>the maximum PS II turnover rate. Modelli<br>increased in parallel with the reversible  $q_1$ .

**Keywords:** photosynthesis; photosystem II; quenching; photoregulation; photoinhibition

### **1. QUENCHING EXCITATION**

Excess light is a frequent problem for upper leaves of plant canopies. Light is in excess when the next photon arrives Excess light is a frequent problem for upper leaves of plant<br>canopies. Light is in excess when the next photon arrives<br>before the electron  $(e^-)$ , produced by the preceding<br>photon has been removed from the photosystem  $H(PST$ canopies. Light is in excess when the next photon arrives<br>before the electron (e<sup>-</sup>), produced by the preceding<br>photon, has been removed from the photosystem II (PS II)<br>acceptor  $\Omega$  (or generally from the terminal accept before the electron (e<sup>-</sup>), produced by the preceding<br>photon, has been removed from the photosystem II (PS II)<br>acceptor  $Q_A$  (or, generally, from the terminal acceptor<br>compound of  $e^-$  transport chain) blocking the way fo photon, has been removed from the photosystem II (PS II)<br>acceptor  $Q_A$  (or, generally, from the terminal acceptor<br>compound of  $e^-$  transport chain) blocking the way for the<br>next  $e^-$ . Thus excess light is a result of imba acceptor  $Q_A$  (or, generally, from the terminal acceptor<br>compound of  $e^-$  transport chain) blocking the way for the<br>next  $e^-$ . Thus, excess light is a result of imbalance between<br>excitation arrival rate in the reaction ce compound of  $e^-$  transport chain) blocking the way for the<br>next  $e^-$ . Thus, excess light is a result of imbalance between<br>excitation arrival rate in the reaction centre and  $e^-$  use<br>rate for CO assimilation, and may appea next  $e^-$ . Thus, excess light is a result of imbalance between<br>excitation arrival rate in the reaction centre and  $e^-$  use<br>rate for  $CO_2$  assimilation, and may appear as a result of<br>too bigh light intensity or too low pho excitation arrival rate in the reaction centre and  $e^-$  use<br>rate for  $CO_2$  assimilation, and may appear as a result of<br>too high light intensity or too low photosynthetic rate<br>(Osmond 1994: Anderson *et al.* 1997: Osmond rate for CO<sub>2</sub> assimilation, and may appear as a result of<br>too high light intensity or too low photosynthetic rate<br>(Osmond 1994; Anderson *et al.* 1997; Osmond *et al.* 1999).<br>With reduced O. PS II is unable to carry out s too high light intensity or too low photosynthetic rate<br>(Osmond 1994; Anderson *et al.* 1997; Osmond *et al.* 1999).<br>With reduced  $Q_A$ , PS II is unable to carry out stable<br>charge senaration i.e. the PS II reaction centres (Osmond 1994; Anderson *et al.* 1997; Osmond *et al.* 1999).<br>With reduced  $Q_A$ , PS II is unable to carry out stable<br>charge separation, i.e. the PS II reaction centres are<br>closed. As a result, the average lifetime of excit With reduced  $Q_A$ , PS II is unable to carry out stable<br>charge separation, i.e. the PS II reaction centres are<br>closed. As a result, the average lifetime of excitation<br>increases from  $300-400$  ps in the state of oxidized O charge separation, i.e. the PS II reaction centres are<br>closed. As a result, the average lifetime of excitation<br>increases from 300-400 ps in the state of oxidized  $Q_A$  to<br> $1-2$  ps in the state of reduced  $Q_A$  (Horton & Rub closed. As a result, the average lifetime of excitation<br>increases from 300–400 ps in the state of oxidized  $Q_A$  to<br>1–2 ns in the state of reduced  $Q_A$  (Horton & Ruban 1994). Such long-living high-energy state, a state of high excita- $1-2$  ns in the state of reduced  $Q_A$  (Horton & Ruban 1994).<br>Such long-living high-energy state, a state of high excita-<br>tion pressure (Gray *et al.* 1996), may be dangerous for the<br>photosynthetic machinery. Nature has wo Such long-living high-energy state, a state of high excitation pressure (Gray *et al.* 1996), may be dangerous for the photosynthetic machinery. Nature has worked out ways to quench this state non-nhotochemically tion pressure (Gray *et al.* 1996), may be ophotosynthetic machinery. Nature has we<br>quench this state non-photochemically. quench this state non-photochemically.<br>(a) **Photochemical quenching** 

In photosynthesis photons excite chlorophyll that forms an antenna system around PS II (Dainese *et al*. 1992;

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Green & Durnford 1996) and the excitation is rapidly transferred to a special pair of chlorophylls  $P_{680}$  in the PS II core complex (Krause & Weis 1991; Renger 1992; transferred to a special pair of chlorophylls  $P_{680}$  in the<br>PS II core complex (Krause & Weis 1991; Renger 1992;<br>Van Grondelle *et al.* 1994; Lavergne & Trissl 1995). Within<br>less than 3 ps a primary radical pair  $P^+$  P **PS II** core complex (Krause & Weis 1991; Renger 1992; Van Grondelle *et al.* 1994; Lavergne & Trissl 1995). Within less than 3 ps a primary radical pair  $P_{680}^+$ Pheo<sup>-</sup> is formed (Wasielewski *et al.* 1989; Iankowiak Van Grondelle *et al.* 1994; Lavergne & Trissl 1995). Within<br>less than 3 ps a primary radical pair  $P_{680}^{+}$ Pheo<sup>-</sup> is formed<br>(Wasielewski *et al.* 1989; Jankowiak *et al.* 1989), but this less than 3 ps a primary radical pair  $P_{680}^{+}$ Pheo<sup>-</sup> is formed (Wasielewski *et al.* 1989; Jankowiak *et al.* 1989), but this state may reverse, and excitation is rapidly transferred to (Wasielewski *et al.* 1989; Jankowiak *et al.* 1989), but this state may reverse, and excitation is rapidly transferred to centre chlorophylls  $P_{680}$  and from it to the antenna (Roelofs *et al.* 1992). Excitation travel state may reverse, and excitation is rapidly transferred to<br>centre chlorophylls  $P_{680}$  and from it to the antenna<br>(Roelofs *et al.* 1992). Excitation travels around the<br>antenna within a picosecond and visits  $P_{\text{tot}}$  a centre chlorophylls  $P_{680}$  and from it to the antenna (Roelofs *et al.* 1992). Excitation travels around the antenna within a picosecond and visits  $P_{680}$  again and again. Each time the  $P^+$  Pheo pair is formed and (Roelofs *et al.* 1992). Excitation travels around the antenna within a picosecond and visits  $P_{680}$  again and again. Each time, the  $P_{680}^+$  Pheo pair is formed and reversed until finally the senarated charges become antenna within a picosecond and visits  $P_{680}$  again and<br>again. Each time, the  $P_{680}^+$  Pheo pair is formed and<br>reversed until finally the separated charges become stabi-<br>lized when  $e^-$  passes from Pheo<sup>-</sup> to the prim again. Each time, the  $P_{680}^{+}$  Pheo pair is formed and<br>reversed until finally the separated charges become stabi-<br>lized when  $e^-$  passes from Pheo<sup>-</sup> to the primary quinone<br>acceptor (Q<sub>A</sub>), which happens within  $350 \pm$ lized when  $e^-$  passes from  $Pheo^-$  to the primary quinone lized when  $e^-$  passes from Pheo<sup>-</sup> to the primary quinone<br>acceptor  $(Q_A)$ , which happens within  $350 \pm 100$  ps<br>(Eckert *et al.* 1988). With oxidized  $Q_A$ , the lifetime of<br>excitation is determined mainly by the time needed acceptor  $(Q_A)$ , which happens within  $350 \pm 100$  ps<br>(Eckert *et al.* 1988). With oxidized  $Q_A$ , the lifetime of<br>excitation is determined mainly by the time needed for<br>charge transfer from Pheo<sup>-</sup> to O<sub>1</sub> termed 'charge st (Eckert *et al.* 1988). With oxidized  $Q_A$ , the lifetime of excitation is determined mainly by the time needed for charge stabi-<br>charge transfer from Pheo<sup>-</sup> to  $Q_A$ , termed 'charge stabi-<br>lization'. When  $Q_A$  is reduced excitation is determined mainly by the time needed for<br>charge transfer from Pheo<sup>-</sup> to  $Q_A$ , termed 'charge stabilization'. When  $Q_A$  is reduced, charge stabilization<br>cannot happen and excitation continues to travel in th charge transfer from Pheo<sup>-</sup> to  $Q_A$ , termed 'charge stabi-<br>lization'. When  $Q_A$  is reduced, charge stabilization<br>cannot happen and excitation continues to travel in the<br>antenna visiting  $P_{xx}$  and trying to form the reve lization. When  $Q_A$  is reduced, charge stabilization<br>cannot happen and excitation continues to travel in the<br>antenna, visiting  $P_{680}$  and trying to form the reversible<br>primary radical pair but failing to because the cha cannot happen and excitation continues to travel in the<br>antenna, visiting  $P_{680}$  and trying to form the reversible<br>primary radical pair, but failing to because the charged<br> $Q^-$  seems to push the next  $e^-$  back by its el  $Q_A^-$  seer tenna, visiting  $P_{680}$  and trying to form the reversible<br>imary radical pair, but failing to because the charged<br> $\bar{A}$  seems to push the next e<sup>-</sup> back by its electrical field,<br>really lowering the average redox potenti primary radical pair, but failing to because the charged  $Q_A^-$  seems to push the next  $e^-$  back by its electrical field, formally lowering the average redox potential of the primary pair state (Van Mieghem *et al.* 1995)  $Q_A^-$  seems to push the next  $e^-$  back by its electrical field,<br>formally lowering the average redox potential of the<br>primary pair state (Van Mieghem *et al.* 1995). Thus, in<br>the presence of reduced  $Q_+$ , the lifetime of formally lowering the average redox potential of the primary pair state (Van Mieghem *et al.* 1995). Thus, in the presence of reduced  $Q_A$ , the lifetime of excitation is determined by two other processes competing for exc primary pair state (Van Mieghem *et al.* 1995). Thus, in<br>the presence of reduced  $Q_A$ , the lifetime of excitation is<br>determined by two other processes competing for excita-<br>tion quenching: dissinative (thermal) conversion the presence of reduced  $Q_A$ , the lifetime of excitation is<br>determined by two other processes competing for excita-<br>tion quenching: dissipative (thermal) conversion and<br>fluorescence emission. Since these quenchers are slo determined by two other processes competing for excitation quenching: dissipative (thermal) conversion and fluorescence emission. Since these quenchers are slower

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than the photochemical charge separation, the lifetime of excitation lengthens approximately five times to *ca*. 2 ns when  $\Omega$ , is reduced than the photochemica<br>excitation lengthens ap<br>when  $Q_A$  is reduced. when  $Q_A$  is reduced.<br> **(b)** *Chlorophyll fluorescence* 

(b) **Chlorophyll fluorescence**<br>The emission of excitation as fluorescence occurs with<br>an approximately constant probability density, i.e. in each<br>time interval the probability of conversion of the excita-The emission of excitation as fluorescence occurs with<br>an approximately constant probability density, i.e. in each<br>time interval the probability of conversion of the excita-<br>tion to fluorescence is constant. The longer the an approximately constant probability density, i.e. in each<br>time interval the probability of conversion of the excita-<br>tion to fluorescence is constant. The longer the lifetime of<br>excitation, the higher the integrated prob time interval the probability of conversion of the excita-<br>tion to fluorescence is constant. The longer the lifetime of<br>excitation, the higher the integrated probability of fluo-<br>rescence emission. Excitation lifetime is t tion to fluorescence is constant. The longer the lifetime of<br>excitation, the higher the integrated probability of fluo-<br>rescence emission. Excitation lifetime is the longest when<br> $\Omega$  is reduced and correspondingly fluores excitation, the higher the integrated probability of fluorescence emission. Excitation lifetime is the longest when  $Q_A$  is reduced and, correspondingly, fluorescence is maximal being suppressed only by the competing rate rescence emission. Excitation lifetime is the longest when  $Q_A$  is reduced and, correspondingly, fluorescence is maximal, being suppressed only by the competing rate constant for thermal conversion. This maximal fluores- $Q_A$  is reduced and, correspondingly, fluorescence is<br>maximal, being suppressed only by the competing rate<br>constant for thermal conversion. This maximal fluores-<br>cence vield is denoted  $F$ . When  $Q_A$  is oxidized the excimaximal, being suppressed only by the competing rate<br>constant for thermal conversion. This maximal fluores-<br>cence yield is denoted  $F_m$ . When  $Q_A$  is oxidized, the exci-<br>tation lifetime is shortest, being determined mainl constant for thermal conversion. This maximal fluores-<br>cence yield is denoted  $F_m$ . When  $Q_A$  is oxidized, the exci-<br>tation lifetime is shortest, being determined mainly by<br>the rate constant of charge stabilization from t cence yield is denoted  $F_m$ . When  $Q_A$  is oxidized, the excitation lifetime is shortest, being determined mainly by the rate constant of charge stabilization from the state of  $P^+$  /Pheo $^-/Q^-$  to  $P^+$  /Pheo $^0/Q^-$  and f  $\rm P_{680}^+$  /  $\rm I$ The interior is shortest, being determined mainly by<br>the rate constant of charge stabilization from the state of<br> $\frac{1}{680}$ /Pheo<sup>-</sup>/Q<sub>A</sub> to P $\frac{1}{680}$ /Pheo/Q<sub>A</sub>, and fluorescence yield<br>the lowest denoted  $F$ . Any inter the rate constant of charge stabilization from the state of  $P_{680}^{+}/P_{\text{he0}}^{-}/Q_A$  to  $P_{680}^{+}/P_{\text{he0}}/Q_A^{-}$ , and fluorescence yield<br>is the lowest, denoted  $F_o$ . Any intermediate fluorescence<br>vield is a sum of emission  $P_{680}^{+}/P$ heo<sup>-</sup>/Q<sub>A</sub> to  $P_{680}^{+}/P$ heo/Q<sub>A</sub>, and fluorescence yield<br>is the lowest, denoted  $F_o$ . Any intermediate fluorescence the<br>yield is a sum of emissions at the  $F_m$  and  $F_o$  levels from prediction state.  $\bullet$  is the lowest, denoted  $F_o$ . Any intermediate fluorescence<br>yield is a sum of emissions at the  $F_m$  and  $F_o$  levels from<br>different PS II, dependent on their  $Q_A$  reduction state.<br>Thus, steady-state fluorescence yield yield is a sum of emissions at the  $F_m$  and  $F_o$  levels from different PS II, dependent on their  $Q_A$  reduction state.<br>Thus, steady-state fluorescence yield  $(F)$  is an indicator of the average lifetime of excitation befor different PS II, dependent on their  $Q_A$  reduction state. Thus, steady-state fluorescence yield  $\langle F \rangle$  is an indicator of<br>the average lifetime of excitation before it becomes<br>quenched, either by photochemical charge separation or<br>by thermal conversion or is emitted as fluoresce the average lifetime of excitation before it becomes<br>quenched, either by photochemical charge separation or<br>by thermal conversion, or is emitted as fluorescence (for a<br>review see I average & Trissl 1995) quenched, either by photochemical c<br>by thermal conversion, or is emitted a<br>review, see Lavergne & Trissl 1995). **(c)** *Non-photochemical quenching*

(c) Non-photochemical quenching<br>Returning to the situation of excess light,  $Q_A$  is<br>duced in most PS II and the fluorescence vield  $(F)$  is (c) **Non-photochemical quenching**<br>Returning to the situation of excess light,  $Q_A$  is<br>reduced in most PS II and the fluorescence yield  $\langle F \rangle$  is<br>expected to be close to  $F$ . Intuitively this is probably a Returning to the situation of excess light,  $Q_A$  is<br>reduced in most PS II and the fluorescence yield (*F*) is<br>expected to be close to  $F_m$ . Intuitively, this is probably a<br>dangerous situation because chlorophylls carrying reduced in most PS II and the fluorescence yield  $\langle F \rangle$  is<br>expected to be close to  $F_m$ . Intuitively, this is probably a<br>dangerous situation because chlorophylls carrying long-<br>living excitation are strong reducers and u expected to be close to  $F_m$ . Intuitively, this is probably a dangerous situation because chlorophylls carrying long-<br>living excitation are strong reducers and uncontrolled<br> $e^-$  transfer to linids or other acceptors may o dangerous situation because chlorophylls carrying long-<br>living excitation are strong reducers and uncontrolled<br> $e^-$  transfer to lipids or other acceptors may occur. Such a<br>high-fluorescence state was observed by H. Kautsk living excitation are strong reducers and uncontrolled e<sup>-</sup> transfer to lipids or other acceptors may occur. Such a<br>high-fluorescence state was observed by H. Kautsky, who<br>saw strong red fluorescence of the leaf when it was<br>suddenly illuminated with blue light Unexpectedly wit high-fluorescence state was observed by H. Kautsky, who<br>saw strong red fluorescence of the leaf when it was<br>suddenly illuminated with blue light. Unexpectedly, within<br>a minute or two the fluorescence weakened to a low leve saw strong red fluorescence of the leaf when it was<br>suddenly illuminated with blue light. Unexpectedly, within<br>a minute or two, the fluorescence weakened to a low level,<br>a phenomenon now, termed the 'Kautsky effect' suddenly illuminated with blue light. Unexpectedly, within<br>a minute or two, the fluorescence weakened to a low level,<br>a phenomenon now termed the `Kautsky effect'<br>(Lichtenthaler 1992: Govindiee 1995) The Kautsky effect a minute or two, the fluorescence weakened to a low level,<br>a phenomenon now termed the 'Kautsky effect'<br>(Lichtenthaler 1992; Govindjee 1995). The Kautsky effect<br>shows that when photochemical quenching is insufficient a phenomenon now termed the 'Kautsky effect' (Lichtenthaler 1992; Govindjee 1995). The Kautsky effect shows that when photochemical quenching is insufficient, the excess excitation must be quenched by another, non-(Lichtenthaler 1992; Govindjee 1995). The Kautsky effect shows that when photochemical quenching is insufficient,<br>the excess excitation must be quenched by another, non-<br>photochemical quencher  $(q_N)$ . Consequently, the fluores-<br>cence level (the average lifetime of excitation) st the excess excitation must be quenched by another, non-<br>photochemical quencher  $(q_N)$ . Consequently, the fluores-<br>cence level (the average lifetime of excitation) stays more<br>or less constant. Since a is complementary to th photochemical quencher  $(q_N)$ . Consequently, the fluorescence level (the average lifetime of excitation) stays more<br>or less constant. Since  $q_N$  is complementary to the photo-<br>chemical quencher  $q_N$  (Laisk *et al.* 1997) t cence level (the average<br>or less constant. Since  $q_1$ <br>chemical quencher  $q_p$  (L<br>these two mechanisms of The central of the average lifetime of excitation) stays more<br>or less constant. Since  $q_N$  is complementary to the photo-<br>- chemical quencher  $q_P$  (Laisk *et al.* 1997), this suggests that<br>these two mechanisms of excitati or less constant. Since  $q_N$  is complementary to the photo-<br>chemical quencher  $q_P$  (Laisk *et al.* 1997), this suggests that<br>these two mechanisms of excitation quenching are similar.<br>The mechanism of non-photochemical que emical quencher  $q_P$  (Laisk *et al.* 1997), this suggests that ese two mechanisms of excitation quenching are similar.<br>The mechanism of non-photochemical quenching has en subject to numerous studies. At first glance, it s

The mechanism of non-photochemical quenching has<br>been subject to numerous studies. At first glance, it seems been subject to numerous studies. At first glance, it seems<br>to be a physiologically important protective mechanism<br>that keeps the excitation lifetime constant independent of<br>the presence or absence of excess light. However to be a physiologically important protective mechanism<br>that keeps the excitation lifetime constant independent of<br>the presence or absence of excess light. However, when<br>present for a long time the initially rapidly reversi that keeps the excitation lifetime constant independent of<br>the presence or absence of excess light. However, when<br>present for a long time the initially rapidly reversible<br>process ('photoprotection state', Osmond et al. 19 the presence or absence of excess light. However, when<br>present for a long time the initially rapidly reversible<br>process ('photoprotection state', Osmond *et al.* 1999)<br>becomes irreversible and continues quenching after the present for a long time the initially rapidly reversible<br>process ('photoprotection state', Osmond *et al.* 1999)<br>becomes irreversible and continues quenching after the<br>light intensity has decreased unnecessarily losing qua process ('photoprotection state', Osmond *et al.* 1999) becomes irreversible and continues quenching after the light intensity has decreased, unnecessarily losing quanta. This situation is termed 'photoiphibition' or 'phot becomes irreversible and continues quenching after the light intensity has decreased, unnecessarily losing quanta.<br> $\perp$ This situation is termed 'photoinhibition' or 'photo-<br>o inactivation' (Osmond 1994; Osmond *et al.* 1 light intensity has decreased, unnecessarily losing quanta. This situation is termed 'photoinhibition' or 'photo- mechanism predicts that no changes occur in PS II inactivation' (Osmond 1994; Osmond *et al.* 1999). Under centres when  $q_E$  is induced.<br>natural conditions where light may protect the photosynthetic machinery when light is natural conditions where light is variable, photoinhibition<br>may protect the photosynthetic machinery when light is<br>high, but it causes loss of valuable quanta when light is<br>low. An ideal photoprotective system would relay may protect the photosynthetic machinery when light is<br>high, but it causes loss of valuable quanta when light is<br>low. An ideal photoprotective system would relax rapidly,<br>following the time-course of the natural variabilit high, but it causes loss of valuable quanta when light is<br>low. An ideal photoprotective system would relax rapidly,<br>following the time-course of the natural variability of *Phil. Trans. R. Soc. Lond.* B (2000)

(b) **Chlorophyll fluorescence** time but it loses this ability when excess light has been<br>The emission of excitation as fluorescence occurs with present for longer time? These questions have been the  $\frac{1}{2}$ <br>light, but the mechanism of  $q_N$  fails to do this. What<br>hannens in PS II or in the light-harvesting antenna when light, but the mechanism of  $q_N$  fails to do this. What<br>happens in PS II or in the light-harvesting antenna when<br> $q_N$  is induced? Why is  $q_N$  able to follow fast changes in light, but the mechanism of  $q_N$  fails to do this. What<br>happens in PS II or in the light-harvesting antenna when<br> $q_N$  is induced? Why is  $q_N$  able to follow fast changes in<br>light intensity when excess light has been prese happens in PS II or in the light-harvesting antenna when  $q_N$  is induced? Why is  $q_N$  able to follow fast changes in light intensity when excess light has been present for short time but it loses this ability when excess  $q_N$  is induced? Why is  $q_N$  able to follow fast changes in light intensity when excess light has been present for short time but it loses this ability when excess light has been present for longer time? These questions h light intensity when excess light has been present for short time but it loses this ability when excess light has been<br>present for longer time? These questions have been the<br>centre of attention for some time. An answer to the first<br>question is contributed here showing that some typ present for longer time? These questions have been the centre of attention for some time. An answer to the first question is contributed here, showing that some types of  $q_N$  are accompanied by changes in  $PS$  H properties centre of attention for some time. An answer to the first<br>question is contributed here, showing that some types of  $q_N$ <br>are accompanied by changes in PS II properties but<br>others are not question is contributed here, showing that some types of  $q_N$  are accompanied by changes in PS II properties but others are not.

### **(d)** *Non-photochemical `energy-dependent' quenching*

(d) *Non-photochemical 'energy-dependent'*<br>quenching<br>The general process of non-photochemical quenching<br>seems to have no single mechanism, but is a succession **quenching**<br>The general process of non-photochemical quenching  $q_N$  seems to have no single mechanism, but is a succession<br>of different processes developing in time. One component The general process of non-photochemical quenching  $q_N$  seems to have no single mechanism, but is a succession of different processes developing in time. One component of the non-photochemical quenching termed 'energy $q_N$  seems to have no single mechanism, but is a succession<br>of different processes developing in time. One component<br>of the non-photochemical quenching, termed 'energy-<br>dependent' quenching  $(a_+)$  is the fastest. It is ind of different processes developing in time. One component<br>of the non-photochemical quenching, termed 'energy-<br>dependent' quenching  $(q_E)$ , is the fastest. It is induced by<br>the acidification of the thulakoid lumen, dependent of the non-photochemical quenching, termed 'energy-<br>dependent' quenching  $(q_E)$ , is the fastest. It is induced by<br>the acidification of the thylakoid lumen, dependent on the<br>presence of antheraxanthin and zeavanthin (Krause dependent' quenching  $(q_E)$ , is the fastest. It is induced by<br>the acidification of the thylakoid lumen, dependent on the<br>presence of antheraxanthin and zeaxanthin (Krause & the acidification of the thylakoid lumen, dependent on the<br>presence of antheraxanthin and zeaxanthin (Krause &<br>Weis 1991; Horton *et al.* 1996). It does this within 30–60 s<br>and is reversed within 5–15 min, but the componen presence of antheraxanthin and zeaxanthin (Krause & Weis 1991; Horton *et al.* 1996). It does this within 30–60s and is reversed within 5–15 min, but the component develops and relaxes more slowly than  $\Delta nH$  itself. This Weis 1991; Horton *et al.* 1996). It does this within 30–60s and is reversed within 5–15 min, but the component develops and relaxes more slowly than  $\Delta pH$  itself. This shows that the process is related to some basic rea and is reversed within 5–15 min, but the component develops and relaxes more slowly than  $\Delta pH$  itself. This develops and relaxes more slowly than  $\Delta pH$  itself. This<br>shows that the process is related to some basic rearrange-<br>ments in the organization of the photosynthetic machinery<br>and the excitation quenching is not a direct con shows that the process is related to some basic rearrange-<br>ments in the organization of the photosynthetic machinery<br>and the excitation quenching is not a direct consequence<br>of proton accumulation or protein protonation ments in the organization of the photosynthetic machinery<br>and the excitation quenching is not a direct consequence<br>of proton accumulation or protein protonation. d the excitation quenching is not a direct consequence<br>proton accumulation or protein protonation.<br>Initially it was proposed that non-photochemical exci-<br>ion quenching occurs in PS II reaction centres that

The mechanism of non-photochemical quenching has ophylls as a result of ligand protonation (Crofts & Yerkes<br>been subject to numerous studies. At first glance, it seems 1994) or between chlorophylls or chlorophyll and<br>Uto b of proton accumulation or protein protonation.<br>
Initially it was proposed that non-photochemical excitation quenching occurs in PS II reaction centres that,<br>
under the influence of the low surrounding pH are Initially it was proposed that non-photochemical excitation quenching occurs in PS II reaction centres that, under the influence of the low surrounding pH, are turned into a form that emits very little fluorescence and tation quenching occurs in PS II reaction centres that, under the influence of the low surrounding pH, are turned into a form that emits very little fluorescence and under the influence of the low surrounding pH, are<br>turned into a form that emits very little fluorescence and<br>has reduced photochemical efficiency (Weis & Berry<br>1987) The centre-quenching model would explain the turned into a form that emits very little fluorescence and<br>has reduced photochemical efficiency (Weis & Berry<br>1987). The centre-quenching model would explain the<br>complementation between photochemical and nonhas reduced photochemical efficiency (Weis & Berry<br>1987). The centre-quenching model would explain the<br>complementation between photochemical and non-<br>photochemical excitation quenching if the trapping 1987). The centre-quenching model would explain the complementation between photochemical and non-photochemical excitation quenching if the trapping constant does not change and the trapped excitation is complementation between photochemical and non-<br>photochemical excitation quenching if the trapping<br>constant does not change and the trapped excitation is<br>dissipated as heat. However, the centre-quenching model photochemical excitation quenching if the trapping constant does not change and the trapped excitation is<br>dissipated as heat. However, the centre-quenching model<br>has difficulties in explaining the non-photochemical<br>quenching of 'dark' fluorescence  $(F)$ , the role of zeadissipated as heat. However, the centre-quenching model<br>has difficulties in explaining the non-photochemical<br>quenching of 'dark' fluorescence  $(F_o)$ , the role of zea-<br>xanthin that is located in the antenna (Demmig-Adams has difficulties in explaining the non-photochemical<br>quenching of 'dark' fluorescence  $(F_o)$ , the role of zea-<br>xanthin that is located in the antenna (Demmig-Adams<br>et al. 1989: Pfiindel and Bilger 1994) and several other quenching of 'dark' fluorescence  $(F_o)$ , the role of zea-<br>xanthin that is located in the antenna (Demmig-Adams<br>*et al.* 1989; Pfündel and Bilger 1994), and several other<br>kinetic phenomena (Ting & Owens 1994; Horton *et al.* xanthin that is located in the antenna (Demmig-Adams *et al.* 1989; Pfündel and Bilger 1994), and several other kinetic phenomena (Ting & Owens 1994; Horton *et al.* 1994; Pfündel & Bilger 1994). A presently widely accepte kinetic phenomena (Ting & Owens 1994; Horton *et al.* 1994; Pfündel & Bilger 1994). A presently widely accepted mechanism explains  $q_E$  as excitation quenching in the LHC II complexes (Horton *et al.* 1996) or in the 1994; Pfündel & Bilger 1994). A presently widely<br>accepted mechanism explains  $q_E$  as excitation quenching<br>in the LHC II complexes (Horton *et al.* 1996) or in the<br>minor CP24 and CP29 complexes (Crofts & Verkes 1994) accepted mechanism explains  $q_E$  as excitation quenching<br>in the LHC II complexes (Horton *et al.* 1996) or in the<br>minor CP24 and CP29 complexes (Crofts & Yerkes 1994)<br>of the antenna caused by close interaction between chl in the LHC II complexes (Horton  $et$   $al.$  1996) or in the minor CP24 and CP29 complexes (Crofts & Yerkes 1994) 1994) or between chlorophylls or chlorophyll and ophylls as a result of ligand protonation (Crofts & Yerkes 1994) or between chlorophylls or chlorophyll and zeaxanthin, which are moved into close contact as a result of protein protonation (Horton *et al* 1994–1996) 1994) or between chlorophylls or chlorophyll and zeaxanthin, which are moved into close contact as a result of protein protonation (Horton *et al.* 1994, 1996). The most convincing evidence that *a* is an antennazeaxanthin, which are moved into close contact as a<br>result of protein protonation (Horton *et al.* 1994, 1996).<br>The most convincing evidence that  $q_E$  is an antenna-<br>hased phenomenon and does not involve charge recomresult of protein protonation (Horton *et al.* 1994, 1996).<br>The most convincing evidence that  $q_E$  is an antenna-<br>based phenomenon and does not involve charge recom-The most convincing evidence that  $q_E$  is an antenna-<br>based phenomenon and does not involve charge recom-<br>bination processes is probably the demonstration that<br> $q_E$  was maintained when leaves were cooled to 77 K based phenomenon and does not involve charge recombination processes is probably the demonstration that  $q_E$  was maintained when leaves were cooled to 77 K<br>(Ruban *et al.* 1993). This photoprotective antenna-based bination processes is probably the demonstration that  $q_E$  was maintained when leaves were cooled to 77 K (Ruban *et al.* 1993). This photoprotective antenna-based mechanism predicts that no changes occur in PS II  $q_E$  was maintained when leaves were cooled to 77 K (Ruban *et al.* 1993). This ph<br>mechanism predicts that n<br>centres when  $q_E$  is induced.

### **(e)** *Very slowly reversible non-photochemical quenching*

When leaves are exposed to the high light for a longer time, the relaxation of  $q_E$  becomes slower and, finally, a

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rather stable situation is created, termed `photoinhibition' (*q*I) (Horton & Ruban 1994) or `photoinactivation' rather stable situation is created, termed 'photoinhibition'  $(q_1)$  (Horton & Ruban 1994) or 'photoinactivation' (Osmond *et al.* 1999). Photoinhibition needs hours or days for relaxation and it is related to some damage  $(q_1)$  (Horton & Ruban 1994) or 'photoinactivation' (Osmond *et al.* 1999). Photoinhibition needs hours or days for relaxation and it is related to some damage in the PS II centres A very slow component of  $q_1$ , the very (Osmond *et al.* 1999). Photoinhibition needs hours or days<br>for relaxation and it is related to some damage in the<br>PS II centres. A very slow component of  $q_N$ , the very<br>slowly reversible photoinhibitory quenching  $q_2$ , for relaxation and it is related to some damage in the<br>PS II centres. A very slow component of  $q_N$ , the very<br>slowly reversible photoinhibitory quenching  $q_L$ , has been<br>subject to thereugh investigation. PS II centres are PS II centres. A very slow component of  $q_N$ , the very slowly reversible photoinhibitory quenching  $q_L$  has been subject to thorough investigation. PS II centres are inactivated in the presence of this type of  $q_L$  as sho slowly reversible photoinhibitory quenching  $q<sub>L</sub>$  has been<br>subject to thorough investigation. PS II centres are<br>inactivated in the presence of this type of  $q<sub>L</sub>$  as shown by<br>the measurements of  $Q<sub>L</sub>$  evolution subject to thorough investigation. PS II centres are<br>inactivated in the presence of this type of  $q_L$ , as shown by<br>the measurements of  $O_2$  evolution from trains of satur-<br>ating single turnover flashes in photoinhibited inactivated in the presence of this type of  $q_L$  as shown by<br>the measurements of  $O_2$  evolution from trains of satur-<br>ating single turnover flashes in photoinhibited leaves<br>(Anderson *et al.* 1995) There is no clear cons the measurements of  $O_2$  evolution from trains of saturating single turnover flashes in photoinhibited leaves (Anderson *et al.* 1995). There is no clear consensus about the mechanism of photoinactivation. One line of ev ating single turnover flashes in photoinhibited leaves<br>(Anderson *et al.* 1995). There is no clear consensus about<br>the mechanism of photoinactivation. One line of evidence<br>emphasizes the possibility that this *a*, origina (Anderson *et al.* 1995). There is no clear consensus about turned off due to the adenylate charge that can reversibly the mechanism of photoinactivation. One line of evidence energize thylakoids (Gilmore & Björkman 1994 the mechanism of photoinactivation. One line of evidence<br>emphasizes the possibility that this  $q_1$  originates from<br>damage in the water-splitting mechanism, while others<br>emphasize changes on the acceptor side, probably du emphasizes the possibility that this  $q_I$  originates from<br>damage in the water-splitting mechanism, while others<br>emphasize changes on the acceptor side, probably due to<br>the double reduction of  $\Omega$ , and the following proto damage in the water-splitting mechanism, while others<br>emphasize changes on the acceptor side, probably due to<br>the double reduction of  $Q_A$  and the following protonation<br>and dissociation of this  $e^-$  carrier (Telfer & Barb emphasize changes on the acceptor side, probably due to<br>the double reduction of  $Q_A$  and the following protonation<br>and dissociation of this e<sup>-</sup> carrier (Telfer & Barber 1994;<br>Styring & Jegerschöld 1994; Zer et al. 1994; the double reduction of  $Q_A$  and the following protonation<br>and dissociation of this  $e^-$  carrier (Telfer & Barber 1994;<br>Styring & Jegerschöld 1994; Zer *et al.* 1994; Ohad *et al.*<br>1994). Both mechanisms are thought to le Styring & Jegerschöld 1994; Zer *et al.* 1994; Ohad *et al.* 1994). Both mechanisms are thought to lead to the degradation of the  $D_1$  protein of the PS II core complex, 1994). Both mechanisms are thought to lead to the degradation of the  $D_1$  protein of the PS II core complex, reparation of which needs the transportation of the narticular PS II into the non-annessed thylakoid region dation of the  $D_1$  protein of the PS II core complex,<br>reparation of which needs the transportation of the<br>particular PS II into the non-appressed thylakoid region,<br>but the causal relationships between photoinhibition and reparation of which needs the transportation of the particular PS II into the non-appressed thylakoid region, but the causal relationships between photoinhibition and D decradation are not completely clear (Obad *et al.* particular PS II into the non-appressed thylakoid region,<br>but the causal relationships between photoinhibition and<br> $D_1$  degradation are not completely clear (Ohad *et al.* 1994;<br>Critchley 1994). Repair of  $D_1$  is a slow but the causal relationships between photoinhibition and  $D_1$  degradation are not completely clear (Ohad *et al.* 1994; Critchley 1994). Repair of  $D_1$  is a slow process and, as the half-time of  $D_1$  degradation is abo  $D_1$  degradation are not completely clear (Ohad *et al.* 1994;<br>Critchley 1994). Repair of  $D_1$  is a slow process and, as the<br>half-time of  $D_1$  degradation is about 1-2 h and repair is<br>even slower (Aro *et al.* 1993), p Critchley 1994). Repair of  $D_1$  is a slow process and, as the half-time of  $D_1$  degradation is about  $1-2h$  and repair is even slower (Aro *et al.* 1993), photoinactivation associated with  $D_1$  degradation certainly ne half-time of  $D_1$  degradation is about  $1-2$  h and repair is<br>even slower (Aro *et al.* 1993), photoinactivation associated<br>with  $D_1$  degradation certainly needs longer than 30 min<br>for relaxation even slower (Archaeology 1)<br>with  $D_1$  degradation.<br>The mechanism th  $D_1$  degradation certainly needs longer than 30 min<br>
The mechanism of  $q_1$  may be related to damage on the<br>
i II donor side or acceptor side but an important

for relaxation.<br>The mechanism of  $q_1$  may be related to damage on the<br>PS II donor side or acceptor side, but an important<br>feature of this damage is that it causes excitation (fluores-The mechanism of  $q_I$  may be related to damage on the<br>PS II donor side or acceptor side, but an important<br>feature of this damage is that it causes excitation (fluores-<br>cence) quenching. In partially photoinhibited leaves PS II donor side or acceptor side, but an important feature of this damage is that it causes excitation (fluorescence) quenching. In partially photoinhibited leaves quenching of both  $a$ , and  $a$ , type is complementary to feature of this damage is that it causes excitation (fluorescence) quenching. In partially photoinhibited leaves quenching of both,  $q_E$  and  $q_I$  type is complementary to  $q_{\text{el}}$ : as much the quantum vield of  $q_{\text{el}}$   $q_P$ : as much the quantum yield of  $q_P$  decreases as much<br>the quantum yield of  $q_E$  or  $q_I$  increases. Consequently the<br>lifetime of excitation remains practically constant (Laisk<br>et al. 1997). This is unexpected if there nce) quenching. In partially photoinhibited leaves<br>tenching of both,  $q_E$  and  $q_I$  type is complementary to<br>c as much the quantum yield of  $q_P$  decreases as much<br>e quantum yield of  $q_{\text{e}}$  or  $q_{\text{e}}$  increases. Conseq quenching of both,  $q_E$  and  $q_I$  type is complementary to  $q_P$ : as much the quantum yield of  $q_E$  or  $q_I$  increases. Consequently the lifetime of excitation remains practically constant (I aisk the quantum yield of  $q_E$  or  $q_I$  increases. Consequently the lifetime of excitation remains practically constant (Laisk *et al.* 1997). This is unexpected if there are different lifetime of excitation remains practically constant (Laisk *et al.* 1997). This is unexpected if there are different mechanisms for the two processes, and suggests that any a quenched **PS II** emits fluorescence at a level *et al.* 1997). This is unexpected if there are different mechanisms for the two processes, and suggests that any  $q_1$  quenched PS II emits fluorescence at a level close to  $F_{\infty}$  as well as any  $q$ , quenched PS II and mechanisms for the two processes, and suggests that any  $q_I$  quenched PS II emits fluorescence at a level close to  $F_{\infty}$  as well as any  $q_E$  quenched PS II and normal  $q_P$  quenched PS II The coincidence of the quenchin  $q_1$  quenched PS II emits fluorescence at a level close to  $F_{\infty}$  as well as any  $q_E$  quenched PS II and normal  $q_P$  quenched PS II. The coincidence of the quenching level in PS II as well as any  $q_E$  quenched PS II and normal  $q_P$  quenched<br>PS II. The coincidence of the quenching level in PS II<br>centres which have oxidized  $P_{680}$ , which lack  $Q_A$  ( $q_I$ ),<br>which have slightly changed position of chlo PS II. The coincidence of the quenching level in PS II<br>centres which have oxidized  $P_{680}$ , which lack  $Q_A$  ( $q_I$ ),<br>which have slightly changed position of chlorophyll in<br>their antenna ( $q_A$ ) or which quench due to charg centres which have oxidized  $P_{680}$ , which lack  $Q_A$  ( $q_I$ ),<br>which have slightly changed position of chlorophyll in<br>their antenna ( $q_E$ ) or which quench due to charge separa-<br>tion and stabilization on  $Q_A$  ( $q_A$ ) encoura which have slightly changed position of chlorophyll in<br>their antenna  $(q_E)$  or which quench due to charge separa-<br>tion and stabilization on  $Q_A (q_P)$  encourages the search<br>for a single mechanism to explain all three states their antenna  $(q_E)$  or which quench due to charge separation and stabilization on  $Q_A (q_P)$  encourages the search for a single mechanism to explain all three states of PS II quenching. Such a mechanism would be one that is tion and stabilization on  $Q_A$  ( $q_P$ ) encourages the search<br>for a single mechanism to explain all three states of PS II<br>quenching. Such a mechanism would be one that is based<br>on charge separation, as for  $q_P$  but with the for a single mechanism to explain all three states of PS II<br>quenching. Such a mechanism would be one that is based<br>on charge separation, as for  $q<sub>P</sub>$ , but with the following<br>recombination of separated charges in the s quenching. Such a mechanism would be one that is based<br>on charge separation, as for  $q_P$ , but with the following<br>recombination of separated charges in the states of  $q_E$  and<br> $q_I$ .<br>(**f**) *Transition type and reversible inh* 

# *nsition type and reversible inhibii*<br>non-photochemical quenching<br>difficult to quantify and understal (f) Transition type and reversible inhibitory<br>non-photochemical quenching<br>The most difficult to quantify and understand is the

**non-photochemical quenching**<br>The most difficult to quantify and understand is the<br>transition type quenching, a state that is induced more<br>slowly than the thylakoid energization related  $a$ , but is The most difficult to quantify and understand is the transition type quenching, a state that is induced more slowly than the thylakoid energization related  $q_E$  but is still reversible though it relaxes more slowly than transition type quenching, a state that is induced more<br>slowly than the thylakoid energization related  $q_E$  but is<br>still reversible, though it relaxes more slowly than  $q_E$ . One<br>such component of  $q_E$  has been related to slowly than the thylakoid energization related  $q_E$  but is still reversible, though it relaxes more slowly than  $q_E$ . One such component of  $q_N$  has been related to state transitions still reversible, though it relaxes more slowly than  $q_E$ . One<br>such component of  $q_N$  has been related to state transitions<br> $(q_T)$ , during which a part of the PS II antenna detaches<br>and moves to the non-appressed region of such component of  $q_N$  has been related to state transitions  $(q_T)$ , during which a part of the PS II antenna detaches and moves to the non-appressed region of thylakoids (Walters & Horton 1991). This process is thought to  $(q_T)$ , during which a part of the PS II antenna detaches<br>and moves to the non-appressed region of thylakoids<br>(Walters & Horton 1991). This process is thought to<br>balance PS II and PS I excitation rates at low absorbed and moves to the non-appressed region of thylakoids a saturating single turnover flash indicates the number of (Walters & Horton 1991). This process is thought to actively  $O_2$  evolving PS II per leaf area. Since one  $O_$ 

quantum flux density (PAD), when  $q_E$  is not yet active<br>enough due to the lower energization level of thulakoids enough due to the lower energization level of thylakoids quantum flux density (PAD), when  $q_E$  is not yet active<br>enough due to the lower energization level of thylakoids<br>(Allen 1992). The time kinetics of this component of  $q_N$  is<br>intermediate between  $q_T$  and irreversible  $q_T$ enough due to the lower energization level of thylakoids<br>(Allen 1992). The time kinetics of this component of  $q_N$  is<br>intermediate between  $q_E$  and irreversible  $q_L$  and  $q_T$  is<br>specifically activated by limiting PADs. Im (Allen 1992). The time kinetics of this component of  $q_N$  is<br>intermediate between  $q_E$  and irreversible  $q_D$  and  $q_T$  is<br>specifically activated by limiting PADs. Importantly, the<br>mechanism of  $q_T$  predicts no changes eith intermediate between  $q_E$  and irreversible  $q_L$  and  $q_T$  is<br>specifically activated by limiting PADs. Importantly, the<br>mechanism of  $q_T$  predicts no changes either in the<br>number of active PS II or in the PS II turnover rat specifically activated by limiting PADs. Importantly, the explain the type of  $q_N$  that is activated by high light and number of active PS II or in the PS II turnover rate. To explain the type of  $q_N$  that is activated by high light and reverses slower than  $q_E$ , it has been proposed that the sustained  $q_E$  can last for a longer time afte explain the type of  $q_N$  that is activated by high light and<br>reverses slower than  $q_E$ , it has been proposed that the<br>sustained  $q_E$  can last for a longer time after the light is<br>turned off due to the adenylate charge tha reverses slower than  $q_E$ , it has been proposed that the sustained  $q_E$  can last for a longer time after the light is turned off due to the adenylate charge that can reversibly energize thylakoids (Gilmore & Biörkman, 199 sustained  $q_E$  can last for a longer time after the light is turned off due to the adenylate charge that can reversibly energize thylakoids (Gilmore & Björkman 1994*a*,*b*).<br>Again, the mechanism of this sustained  $q_E$  is principally<br>the same as for ordinary  $q_E$  and it also predicts no<br>changes in the number of active PS II Again, the mechanism of this sustained<br>the same as for ordinary  $q_E$  and it<br>changes in the number of active PS II.<br>Although this component of  $q_E$  has e same as for ordinary  $q_E$  and it also predicts no<br>anges in the number of active PS II.<br>Although this component of  $q_N$  has been classified as<br>(Walters & Horton 1991) where the subscript denotes

changes in the number of active PS II.<br>Although this component of  $q_N$  has been classified as  $q_I$  (Walters & Horton 1991), where the subscript denotes photoinhibition, it still is thought to happen in the Although this component of  $q_N$  has been classified as  $q_I$  (Walters & Horton 1991), where the subscript denotes photoinhibition, it still is thought to happen in the antenna because during this phase of  $q_{\text{L}}$  similar  $q_1$  (Walters & Horton 1991), where the subscript denotes photoinhibition, it still is thought to happen in the antenna, because during this phase of  $q_N$  similar changes take place in the antenna as during  $q_2$  quenchi photoinhibition, it still is thought to happen in the antenna, because during this phase of  $q_N$  similar changes take place in the antenna as during  $q_E$  quenching antenna, because during this phase of  $q_N$  similar changes<br>take place in the antenna as during  $q_E$  quenching<br>(Horton & Ruban 1994). However, as Horton and<br>Ruban note this interpretation may be a problem since take place in the antenna as during  $q_E$  quenching<br>(Horton & Ruban 1994). However, as Horton and<br>Ruban note, this interpretation may be a problem since<br>the reversible a can overlap with the irreversible a (Horton & Ruban 1994). However, as Horton and Ruban note, this interpretation may be a problem since the reversible  $q_1$  can overlap with the irreversible  $q_2$  a type of quenching known to occur when PS II is Ruban note, this interpretation may be a problem since<br>the reversible  $q_1$  can overlap with the irreversible  $q_1$ , a<br>type of quenching known to occur when PS II is<br>damaged (Horton & Ruban 1994). This transient type of the reversible  $q_1$  can overlap with the irreversible  $q_1$ , a<br>type of quenching known to occur when PS II is<br>damaged (Horton & Ruban 1994). This transient type of<br>quenching is presently the most controversial. Since it type of quenching known to occur when PS II is cannot be classified as  $q_E$  or  $q_T$ , it is denoted reversible inhibitory non-phytochemical quenching  $(q_I)$ , in accorcannot be classified as  $q_E$  or  $q_T$ , it is denoted reversible<br>inhibitory non-phytochemical quenching  $(q_I)$ , in accor-<br>dance with (Horton & Ruban 1994), but emphasizing<br>the difference from the irreversible (or very slowly inhibitory non-phytochemical quenching  $(q_1)$ , in accordance with (Horton & Ruban 1994), but emphasizing<br>the difference from the irreversible (or very slowly rever-<br>sible)  $q_2$  that can be explained on the basis of possi dance with (Horton & Ruban 1994), but emphasizing<br>the difference from the irreversible (or very slowly rever-<br>sible)  $q_I$  that can be explained on the basis of possible<br>damages in PS II. The experiments below were aimed t the difference from the irreversible (or very slowly reversible)  $q_I$  that can be explained on the basis of possible damages in PS II. The experiments below were aimed to detect whether PS II properties were altered durin sible)  $q_I$  that can be explained on the basis of possible damages in PS II. The experiments below were aimed to detect whether PS II properties were altered during  $q_E$  and reversible  $q_I$ . and reversible  $q_I$ .

### **2. MEASUREMENT OF PS II PROPERTIES**

The measurement of the actual functional state of 2. MEASUREMENT OF PS II PROPERTIES<br>The measurement of the actual functional state of<br>PS II in the presence and absence of different forms of<br>quenching is of crucial importance in the investigation of The measurement of the actual functional state of  $PS~II$  in the presence and absence of different forms of quenching is of crucial importance in the investigation of  $a$ . Measurements carried out on intact leaves guarantee **PS II** in the presence and absence of different forms of quenching is of crucial importance in the investigation of  $q_N$ . Measurements carried out on intact leaves guarantee that no artefacts caused by thylakoid preparat quenching is of crucial importance in the investigation of  $q_N$ . Measurements carried out on intact leaves guarantee that no artefacts caused by thylakoid preparation or by  $q_N$ . Measurements carried out on intact leaves guarantee<br>that no artefacts caused by thylakoid preparation or by<br>interference of inhibitors are introduced into the results.<br>The PS II state can be sensed by  $Q_2$  evolutio that no artefacts caused by thylakoid preparation or by<br>interference of inhibitors are introduced into the results.<br>The PS II state can be sensed by  $O_2$  evolution and by<br>chlorophyll fluorescence. During, these measureme interference of inhibitors are introduced into the results.<br>The PS II state can be sensed by  $O_2$  evolution and by<br>chlorophyll fluorescence. During these measurements,<br>care must be taken not to limit the PS II function b The PS II state can be sensed by  $O_2$  evolution and by<br>chlorophyll fluorescence. During these measurements,<br>care must be taken not to limit the PS II function by<br>blocking  $e^-$  transport on the acceptor side i.e. these chlorophyll fluorescence. During these measurements,<br>care must be taken not to limit the PS II function by<br>blocking e<sup>-</sup> transport on the acceptor side, i.e. these<br>measurements must be carried out with (almost) comcare must be taken not to limit the PS II function by blocking  $e^-$  transport on the acceptor side, i.e. these measurements must be carried out with (almost) comblocking  $e^-$  transport on the acceptor side, i.e. these<br>measurements must be carried out with (almost) com-<br>pletely oxidized plastoquinone (PQ), the terminal<br>acceptor of  $e^-$  from PS II As the PO pool is about  $8$ measurements must be carried out with (almost) completely oxidized plastoquinone (PQ), the terminal acceptor of  $e^-$  from PS II. As the PQ pool is about 8–<br>12 mol per PS II or about 16–24 $e^-$  per PS II the pletely oxidized plastoquinone (PQ), the terminal acceptor of  $e^-$  from PS II. As the PQ pool is about 8–<br>12 mol per PS II, or about 16–24 $e^-$  per PS II, the<br>amount of transferred  $e^-$  is limited to a fraction of this acceptor of  $e^-$  from PS II. As the PQ pool is about 8–<br>12 mol per PS II, or about 16–24 $e^-$  per PS II, the<br>amount of transferred  $e^-$  is limited to a fraction of this.<br>Thus, only short flashes or pulses of light can be u 12 mol per PS II, or about  $16-24e^-$  per PS II, the<br>amount of transferred  $e^-$  is limited to a fraction of this.<br>Thus, only short flashes or pulses of light can be used<br>during which no more than  $4-5e^-$  per PS II are tran amount of transferred  $e^-$  is limited to a fraction of this.<br>Thus, only short flashes or pulses of light can be used<br>during which no more than  $4-5e^-$  per PS II are trans-<br>ferred A longer exposure would inevitably cause P Thus, only short flashes or pulses of light can be used<br>during which no more than  $4-5e^-$  per PS II are trans-<br>ferred. A longer exposure would inevitably cause PQ<br>reduction followed by O, reduction and PS II closure A during which no more than  $4-5e^-$  per PS II are transferred. A longer exposure would inevitably cause PQ reduction, followed by  $Q_A$  reduction and PS II closure. A widely used but still controversial approach is applying ferred. A longer exposure would inevitably cause PQ<br>reduction, followed by  $Q_A$  reduction and PS II closure. A<br>widely used, but still controversial, approach is applying<br>single turnover flashes, which are so short that ev reduction, followed by  $Q_A$  reduction and PS II closure. A widely used, but still controversial, approach is applying single turnover flashes, which are so short that every PS II can turn over only once transporting only widely used, but still controversial, approach is applying<br>single turnover flashes, which are so short that every<br>PS II can turn over only once, transporting only one e<sup>-</sup><br>(Chow et al. 1989–1991). The amount of O<sub>r</sub> evolv single turnover flashes, which are so short that every<br>PS II can turn over only once, transporting only one  $e^-$ <br>(Chow *et al.* 1989, 1991). The amount of  $O_2$  evolved from<br>a saturating single turnover flash indicates th PS II can turn over only once, transporting only one  $e^-$ <br>(Chow *et al.* 1989, 1991). The amount of  $O_2$  evolved from<br>a saturating single turnover flash indicates the number of<br>actively  $O$  evolving PS II per leaf area. (Chow *et al.* 1989, 1991). The amount of  $O_2$  evolved from<br>a saturating single turnover flash indicates the number of<br>actively  $O_2$  evolving PS II per leaf area. Since one  $O_2$ <br>evolves per four transported  $e^-$ , the a a saturating single turnover flash indicates the number of

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Multiple turnover pulses allow several PS II turnovers  $O_2$  per flash equals a maximum of one-quarter PS II.<br>Multiple turnover pulses allow several PS II turnovers<br>and  $O_2$  evolution from a saturating multiple turnover<br>pulse indicates the maximum rate of PS II turnover. Bot Multiple turnover pulses allow several PS II turnovers<br>and  $O_2$  evolution from a saturating multiple turnover<br>pulse indicates the maximum rate of PS II turnover. Both<br>these methods require intense light sources and a sen and  $O_2$  evolution from a saturating multiple turnover<br>pulse indicates the maximum rate of PS II turnover. Both<br>these methods require intense light sources and a sensitive<br>method for  $O_2$  recording pulse indicates the maximum rate of PS II turnover. Both<br>these methods require intense light sources and a sensitive<br>method for  $O_2$  recording.

### **(a)** *Flashes and pulses*

For the measurements described below single turnover (a) *Flashes and pulses*<br>
For the measurements described below single turnover<br>
flashes were produced by a Machine Vision Strobe<br>
MVS-7020 (EG&G Ontoelectronics Salem MA USA) For the measurements described below single turnover<br>flashes were produced by a Machine Vision Strobe<br>MVS-7020 (EG&G Optoelectronics, Salem, MA, USA)<br>with 12 or 4 uE discharge capacitors and applied to the flashes were produced by a Machine Vision Strobe<br>MVS-7020 (EG&G Optoelectronics, Salem, MA, USA)<br>with 12 or 4  $\mu$ F discharge capacitors and applied to the<br>leaf via a branch of the fibre-optic light guide. The dura-MVS-7020 (EG&G Optoelectronics, Salem, MA, USA)<br>with 12 or  $4 \mu$ F discharge capacitors and applied to the<br>leaf via a branch of the fibre-optic light guide. The dura-<br>tion of flashes at half-height was 6 and 3.3 us respecwith 12 or  $4 \mu$ F discharge capacitors and applied to the<br>leaf via a branch of the fibre-optic light guide. The dura-<br>tion of flashes at half-height was 6 and 3.3  $\mu$ s, respec-<br>tively. This flashlamn is equipped with a p leaf via a branch of the fibre-optic light guide. The dura-<br>tion of flashes at half-height was 6 and 3.3 µs, respec-<br>tively. This flashlamp is equipped with a powerful<br>parabolic mirror which allows concentration of most of tion of flashes at half-height was 6 and 3.3 µs, respectively. This flashlamp is equipped with a powerful parabolic mirror, which allows concentration of most of the flash energy into a bundle of fibres. Elash doses of tively. This flashlamp is equipped with a powerful<br>parabolic mirror, which allows concentration of most of<br>the flash energy into a bundle of fibres. Flash doses of<br> $110$  and  $60 \text{ und } m^{-2}$  were obtained in the leaf chamber parabolic mirror, which allows concentration of most of<br>the flash energy into a bundle of fibres. Flash doses of<br>110 and 60 µmol m<sup>-2</sup> were obtained in the leaf chamber<br>with the <sup>12</sup> and 4 uE capacitors, respectively. Fla the flash energy into a bundle of fibres. Flash doses of<br>110 and 60  $\mu$ mol m<sup>-2</sup> were obtained in the leaf chamber<br>with the 12 and 4  $\mu$ F capacitors, respectively. Flashes were<br>attenuated with neutral filters when neces 110 and 60  $\mu$ mol m<sup>-2</sup> were obtained in the leaf chamber<br>with the 12 and  $4 \mu$ F capacitors, respectively. Flashes were<br>attenuated with neutral filters when necessary. The flashes<br>were single turnover, since yery little with the 12 and  $4 \mu$ F capacitors, respectively. Flashes were<br>attenuated with neutral filters when necessary. The flashes<br>were single turnover, since very little  $O_2$  evolution was<br>recorded from the second flash and maxi attenuated with neutral filters when necessary. The flashes<br>were single turnover, since very little  $O_2$  evolution was<br>recorded from the second flash and maximum  $O_2$  evolu-<br>tion occurred in the third flash from a darkwere single turnover, since very little  $O_2$  evolution was<br>recorded from the second flash and maximum  $O_2$  evolu-<br>tion occurred in the third flash from a dark-adapted leaf.<br>Multiple turnover pulses of up to 15.000 umol recorded from the second flash and maximum  $O_2$  evolution occurred in the third flash from a dark-adapted leaf.<br>Multiple turnover pulses of up to  $15\,000$  µmol quanta s is the third flash from a dark-adapted leaf.<br>1971 ultiple turnover pulses of up to  $15000 \mu$ mol quanta<br>1971 were provided by a Schott KL 1500 light source<br>1971 Effektrich Germany) A computer-triggered Multiple turnover pulses of up to  $15\,000\,\mu$ mol quanta<br>m<sup>-2</sup> s<sup>-1</sup> were provided by a Schott KL 1500 light source<br>(H. Walz, Effeltrich, Germany). A computer-triggered<br>spring-operated shutter was constructed (Fast-Est Ta  $\rm{m^{-2} \ s^{-1}}$  were provided by a Schott KL 1500 light source (H. Walz, Effeltrich, Germany). A computer-triggered spring-operated shutter was constructed (Fast-Est, Tartu, Estonia) that fit into the body of the Schott KI (H. Walz, Effeltrich, Germany). A computer-triggered spring-operated shutter was constructed (Fast-Est, Tartu, Estonia) that fit into the body of the Schott KL 1500 electronic light source in the slit of the slide filter h spring-operated shutter was constructed (Fast-Est, Tartu, Estonia) that fit into the body of the Schott KL 1500<br>electronic light source in the slit of the slide filter holder<br>and produced pulses of variable length with edg Estonia) that fit into the body of the Schott KL 1500<br>electronic light source in the slit of the slide filter holder<br>and produced pulses of variable length with edges of 1ms.<br>The time-course of light intensity during pulse and produced pulses of variable length with edges of 1 ms.<br>The time-course of light intensity during pulses was and produced pulses of variable length with edges of 1 ms.<br>The time-course of light intensity during pulses was<br>recorded with a LiCor LI-190SA quantum sensor (LiCor,<br>Lincoln NE, USA), Light from different sources (actinic The time-course of light intensity during pulses was<br>recorded with a LiCor LI-190SA quantum sensor (LiCor,<br>Lincoln, NE, USA). Light from different sources (actinic<br>hackground far-red fluorescence saturation pulses single recorded with a LiCor LI-190SA quantum sensor (LiCor,<br>Lincoln, NE, USA). Light from different sources (actinic<br>background, far-red, fluorescence saturation pulses, single<br>turnover flashes and multiple turnover pulses) was Lincoln, NE, USA). Light from different sources (actinic background, far-red, fluorescence saturation pulses, single turnover flashes and multiple turnover pulses) was evenly superimposed over the leaf area by a multibranc background, far-red, fluorescence saturation pulses, singleturnover flashes and multiple turnover pulses) was evenly against the same quantum sensor in its linear range. Both<br>superimposed over the leaf area by a multibranch fibre-<br>optic light guide. A Schott KL 1500 light source w superimposed over the leaf area by a multibranch fibre-<br>optic light guide. A Schott KL 1500 light source was used<br>for background actinic illumination (to vary  $q_N$ ); for<br>fluorescence saturation pulses the intensity of the optic light guide. A Schott KL 1500 light source was used<br>for background actinic illumination (to vary  $q_N$ ); for<br>fluorescence saturation pulses the intensity of the same<br>source was electronically turned to 15,000 umol m<sup></sup> for background actinic illumination (to vary  $q_N$ ); for fluorescence saturation pulses the intensity of the sam source was electronically turned to 15 000 µmol m<sup>-2</sup>s<sup>-</sup> for 1s. Another Schott KI, 1500, filtered through a source was electronically turned to  $15000 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> fluorescence saturation pulses the intensity of the same<br>source was electronically turned to  $15000 \mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>for 1s. Another Schott KL 1500, filtered through a 720 nm narrow-band interference filter, was used for far-red for 1s. Another Schott KL 1500, filtered through a 720<br>nm narrow-band interference filter, was used for far-red<br>(FR) illumination (incident intensity 240 µmol m<sup>-2</sup>s<sup>-1</sup>).<br>The absorption coefficient of the leaf for photos nm narrow-band interference filter, was used for far-red<br>(FR) illumination (incident intensity 240 µmol m<sup>-2</sup>s<sup>-1</sup>).<br>The absorption coefficient of the leaf for photosyntheti-<br>cally active radiation was measured in an inte (FR) illumination (incident intensity 240  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>).<br>The absorption coefficient of the leaf for photosynthetically active radiation was measured in an integrating<br>sphere Irradiation density is expressed as absor The absorption coefficient of the leaf for photosynthetically active radiation was measured in an integrating sphere. Irradiation density is expressed as absorbed quantum flux density  $(PAD)$ cally active radiation was m<br>sphere. Irradiation density i<br>quantum flux density (PAD). **quantum flux density (PAD).**<br>**(b)** *Measuring the flash dose* 

(b) *Measuring the flash dose*<br>Single turnover flashes average about 3–6 µs long and<br>are very bright. The intensity of illumination during the<br>flash rises to about 2 mol quanta  $m^{-2}s^{-1}$  brighter than a Single turnover flashes average about  $3-6$  µs long and<br>are very bright. The intensity of illumination during the<br>flash rises to about 2 mol quanta m<sup>-2</sup>s<sup>-1</sup>, brighter than a<br>thousand suns. Photoelectric sensors, such as flash rises to about 2 mol quanta m<sup>-2</sup>s<sup>-1</sup>, brighter than a are very bright. The intensity of illumination during the<br>flash rises to about 2 mol quanta  $m^{-2}s^{-1}$ , brighter than a<br>thousand suns. Photoelectric sensors, such as photodiodes,<br>may become nonlinear at such bigh quantum f flash rises to about 2 mol quanta  $m^{-2} s^{-1}$ , brighter than a<br>thousand suns. Photoelectric sensors, such as photodiodes,<br>may become nonlinear at such high quantum flux densi-<br>ties and the flash is so short that its shape thousand suns. Photoelectric sensors, such as photodiodes,<br>may become nonlinear at such high quantum flux densi-<br>ties and the flash is so short that its shape can be recorded<br>only by an oscilloscope. However, measurement o may become nonlinear at such high quantum flux densi-<br>ties and the flash is so short that its shape can be recorded<br>only by an oscilloscope. However, measurement of flash<br>intensity is not as important as its dose, the inte ties and the flash is so short that its shape can be recorded<br>only by an oscilloscope. However, measurement of flash<br>intensity is not as important as its dose, the integral of the<br>flash in quanta per square metre. The inte % only by an oscilloscope. However, measurement of flash in ments is a zirconium oxide  $O_2$  analyser. However, its intensity is not as important as its dose, the integral of the sensitivity meets the above requirements o  $\perp$  intensity is not as important as its dose, the integral of the ments reported below the LiCor quantum sensor LIeasily be done electrically. For example, in the measure-<br>ments reported below the LiCor quantum sensor LI-<br>190SA was used. During the flash-dose measurements the<br>photocurrent of the sensor photodiode connected in the ments reported below the LiCor quantum sensor LI-<br>190SA was used. During the flash-dose measurements the<br>photocurrent of the sensor photodiode, connected in the<br>reverse direction, charged a canacitor of  $10 \text{ uF}$  from a 190SA was used. During the flash-dose measurements the photocurrent of the sensor photodiode, connected in the reverse direction, charged a capacitor of  $10 \,\mu\text{F}$  from a reverse direction, charged a capacitor of  $10 \mu$ F from a *Phil. Trans. R. Soc. Lond.* B (2000)

4.5 V battery. The capacitor was discharged simultaneously through a 0.44  $\text{M}\Omega$  resistor (time constant 4.4 s). 4.5 V battery. The capacitor was discharged simultaneously through a  $0.44 \text{ M}\Omega$  resistor (time constant 4.4 s).<br>Each flash produced a transient, the peak of which was proportional to the pulse quantum dose. The speed of neously through a  $0.44 \text{ M}\Omega$  resistor (time constant 4.4 s).<br>Each flash produced a transient, the peak of which was<br>proportional to the pulse quantum dose. The speed of the<br>transient was such that its neak could be reco Each flash produced a transient, the peak of which was<br>proportional to the pulse quantum dose. The speed of the<br>transient was such that its peak could be recorded by the<br>data logger along with other signals, without the ne proportional to the pulse quantum dose. The speed of the transient was such that its peak could be recorded by the data logger along with other signals, without the necessity for a fast recorder. transient was such that its peak could be recorded by the ta logger along with other signals, without the necessity<br>Flash energy measurements were calibrated in two<br>dependent ways. First, the photodiode of the LL190SA

for a fast recorder.<br>
Flash energy measurements were calibrated in two<br>
independent ways. First, the photodiode of the LI-190SA<br>
sensor was found to be linear up to  $25 \text{ umol}$  quanta m<sup>-2</sup> independent ways. First, the photodiode of the LI-190SA sensor was found to be linear up to 25  $\mu$ mol quanta m<sup>-2</sup> independent ways. First, the photodiode of the LI-190SA<br>sensor was found to be linear up to 25  $\mu$ mol quanta m<sup>-2</sup><br>(the linearity was tested by checking whether the decay of<br>the flash shape recorded on oscilloscope with sensor was found to be linear up to  $25 \mu$ molquanta m<sup>-2</sup><br>(the linearity was tested by checking whether the decay of<br>the flash shape recorded on oscilloscope with the help of<br>the same sensor was still exponential). The in (the linearity was tested by checking whether the decay of<br>the flash shape recorded on oscilloscope with the help of<br>the same sensor was still exponential). The integral of the<br>current during the flash was recalculated int the flash shape recorded on oscilloscope with the help of<br>the same sensor was still exponential). The integral of the<br>current during the flash was recalculated into the same sensor was still exponential). The integral of the current during the flash was recalculated into  $\mu$ mol quanta m<sup>-2</sup> using the calibration constant of the sensor from the supplier. Elash intensity had to be current during the flash was recalculated into  $\mu$ molquanta m<sup>-2</sup> using the calibration constant of the sensor from the supplier. Flash intensity had to be attenuated four times for these measurements. Second a  $\mu$ mol quanta m<sup>-2</sup> using the calibration constant of the sensor from the supplier. Flash intensity had to be attenuated four times for these measurements. Second, a thermoelectric pyranometer was used as an intermediate sensor from the supplier. Flash intensity had to be attenuated four times for these measurements. Second, a thermoelectric pyranometer was used as an intermediate sensor to compare the high-peak but short  $(6 \mu s)$  single attenuated four times for these measurements. Second, a turnover flashes with lower-peak but longer (10 ms) sensor to compare the high-peak but short  $(6 \mu s)$  single<br>turnover flashes with lower-peak but longer  $(10 \text{ ms})$ <br>multiple turnover flashes of similar energy. Responses<br>from a series of  $10 \text{ ms}$  long pulses of different i turnover flashes with lower-peak but longer (10 ms)<br>multiple turnover flashes of similar energy. Responses<br>from a series of 10 ms long pulses of different intensity of<br>up to 15.000 umol m<sup>-2</sup>s<sup>-1</sup> were recorded by the LL1 multiple turnover flasher<br>from a series of 10 ms los<br>up to 15 000 µmol m<sup>-2</sup>s<sup>-</sup> hes of similar energy. Responses<br>long pulses of different intensity of<br> $s^{-1}$  were recorded by the LI-190SA<br>trace recorded with  $40 \text{ us data}$ . from a series of 10 ms long pulses of different intensity of<br>up to 15 000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> were recorded by the LI-190SA<br>quantum sensor (true trace recorded with 40  $\mu$ s data-<br>logging frequency) and by the thermoelect up to 15 000  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> were recorded by the LI-190SA quantum sensor (true trace recorded with 40  $\mu$ s data-logging frequency) and by the thermoelectric pyranoquantum sensor (true trace recorded with 40 us data-<br>logging frequency) and by the thermoelectric pyrano-<br>meter (a slow bell-shaped integral response recorded with<br>l.ms data-logging speed). This way the pyranometer was logging frequency) and by the thermoelectric pyranometer (a slow bell-shaped integral response recorded with 1 ms data-logging speed). This way the pyranometer was calibrated in units of umolquanta  $m^{-2}$  against the meter (a slow bell-shaped integral response recorded with<br>1 ms data-logging speed). This way the pyranometer was<br>calibrated in units of  $\mu$ mol quanta m<sup>-2</sup> against the<br>I L100SA sensor in the range of guaranteed linearity l ms data-logging speed). This way the pyranometer was<br>calibrated in units of  $\mu$ mol quanta  $m^{-2}$  against the<br>LI-190SA sensor in the range of guaranteed linearity of<br>the latter. Then the same procedure was repeated with calibrated in units of  $\mu$ mol quanta  $m^{-2}$  against the LL-190SA sensor in the range of guaranteed linearity of the latter. Then the same procedure was repeated with single turnover flashes recording the integral of flas LI-190SA sensor in the range of guaranteed linearity of the latter. Then the same procedure was repeated with single turnover flashes, recording the integral of flashes the latter. Then the same procedure was repeated with<br>single turnover flashes, recording the integral of flashes<br>by the quantum sensor instead of the true trace. As a<br>result, the flash quantum dosage meter was calibrated single turnover flashes, recording the integral of flashes<br>by the quantum sensor instead of the true trace. As a<br>result, the flash quantum dosage meter was calibrated<br>against the pyrapometer which itself had been calibrate by the quantum sensor instead of the true trace. As a<br>result, the flash quantum dosage meter was calibrated<br>against the pyranometer, which itself had been calibrated<br>against the same quantum sensor in its linear range. Bot result, the flash quantum dosage meter was calibrated<br>against the pyranometer, which itself had been calibrated methods gave similar results, from which it was concluded that the LI-190SA sensor is a linear meter up to methods gave similar results, from which it was concluded that the LI-190SA sensor is a linear meter up to about 25 µmol quanta m<sup>-2</sup> in single turnover flashes (6 µs long on half-height). For measurement of greater doses cluded that the LI-190SA sensor is a linear meter up to about 25  $\mu$ mol quanta m<sup>-2</sup> in single turnover flashes (6  $\mu$ s long on half-height). For measurement of greater doses, the flashes were attenuated with neutral de about 25  $\mu$ mol quanta m<sup>-2</sup> in single turnover flashes (6  $\mu$  long on half-height). For measurement of greater dose<br>the flashes were attenuated with neutral density filters. the flashes were attenuated with neutral density filters.<br>(c) *Oxygen evolution from flashes and pulses* 

(b) **Measuring the flash dose**<br>Single turnover flashes average about 3-6  $\mu$ s long and order the Mn cluster of the oxygen-evolving complex (OEC)<br>Single turnover flashes average about 3-6  $\mu$ s long and are randomized. For The highest sensitivity of  $O_2$  evolution measurements is (c) Oxygen evolution from flashes and pulses<br>The highest sensitivity of O<sub>2</sub> evolution measurements is<br>required with single turnover flashes. In leaves the<br>number of active PS II is usually about  $1-2 \text{ und } m^2$ The highest sensitivity of  $O_2$  evolution measurements is<br>required with single turnover flashes. In leaves the<br>number of active PS II is usually about  $1-2$  µmol m<sup>2</sup><br>(Chow et al. 1989, 1991; figure 2) and correspondingl required with single turnover flashes. In leaves the number of active PS II is usually about  $1-2 \mu$ mol m<sup>2</sup> (Chow *et al.* 1989, 1991; figure 2) and, correspondingly, 0.25–0.5 umol m<sup>-2</sup> is the expected maximum  $Q_1$  evo number of active PS II is usually about  $1-2 \mu$ mol m<sup>2</sup><br>(Chow *et al.* 1989, 1991; figure 2) and, correspondingly,<br>0.25–0.5  $\mu$ mol m<sup>-2</sup> is the expected maximum O<sub>2</sub> evo-<br>lution from one saturating flash when s-states of (Chow *et al.* 1989, 1991; figure 2) and, correspondingly, 0.25–0.5  $\mu$ mol m<sup>-2</sup> is the expected maximum O<sub>2</sub> evolution from one saturating flash when s-states of oxidation of the Mn cluster of the oxygen-evolution compl 0.25–0.5  $\mu$ mol m<sup>-2</sup> is the expected maximum O<sub>2</sub> evolution from one saturating flash when s-states of oxidation of the Mn cluster of the oxygen-evolving complex (OEC) are randomized. For reliable measurement of the fla lution from one saturating flash when s-states of oxidation<br>of the Mn cluster of the oxygen-evolving complex (OEC)<br>are randomized. For reliable measurement of the flash<br>saturation curve, a threshold sensitivity must be 0.0 of the Mn cluster of the oxygen-evolving complex (OEC)<br>are randomized. For reliable measurement of the flash<br>saturation curve, a threshold sensitivity must be  $0.005-$ <br> $0.01 \text{ } \text{mmol O}$  m<sup>-2</sup> requiring an analysis system are randomized. For reliable measurement of the flash<br>saturation curve, a threshold sensitivity must be 0.005–<br>0.01  $\mu$ mol O<sub>2</sub> m<sup>-2</sup>, requiring an analysis system sensitivity<br>of 5–10 pmol O<sub>2</sub> when leaf area is  $10 \text{ cm}$ saturation curve, a threshold sensitivity must be 0.005–<br>0.01  $\mu$ mol O<sub>2</sub> m<sup>-2</sup>, requiring an analysis system sensitivity<br>of 5–10 pmol O<sub>2</sub> when leaf area is 10 cm<sup>2</sup>. Considering<br>that this is still 6.02 × 10<sup>11</sup> molecul 0.01 µmol  $O_2$  m<sup>-2</sup>, requiring an analysis system sensitivity<br>of 5-10 pmol  $O_2$  when leaf area is 10 cm<sup>2</sup>. Considering<br>that this is still 6.02×10<sup>11</sup> molecules of  $O_2$ , the task is not<br>impossible. The most sensitive of 5–10 pmol  $O_2$  when leaf area is 10 cm<sup>2</sup>. Considering<br>that this is still  $6.02 \times 10^{11}$  molecules of  $O_2$ , the task is not<br>impossible. The most sensitive for gas phase measure-<br>ments is a zirconium oxide O analyser that this is still  $6.02 \times 10^{11}$  molecules of  $O_2$ , the task is not impossible. The most sensitive for gas phase measurements is a zirconium oxide  $O_2$  analyser. However, its<br>sensitivity meets the above requirements only when the<br>background  $O_2$  concentration is low, such that the  $O_2$ <br>evolution causes a sufficient relative increase ove sensitivity meets the above requirements only when the<br>background  $O_2$  concentration is low, such that the  $O_2$ <br>evolution causes a sufficient relative increase over the<br>background  $O_2$  concentration (Laisk & Oia 1998) background  $O_2$  concentration is low, such that the  $O_2$ <br>evolution causes a sufficient relative increase over the<br>background  $O_2$  concentration (Laisk & Oja 1998). Low<br>background  $O_2$  concentration is required not onl evolution causes a sufficient relative increase over the<br>background  $O_2$  concentration (Laisk & Oja 1998). Low<br>background  $O_2$  concentration is required not only<br>because of the decreasing sensitivity of the analyser but background  $O_2$  concentration (Laisk & Oja 1998). Low<br>background  $O_2$  concentration is required not only<br>because of the decreasing sensitivity of the analyser but<br>also to keep the background O concentration absolutely background  $O_2$  concentration is required not only<br>because of the decreasing sensitivity of the analyser but<br>also to keep the background  $O_2$  concentration absolutely

*Alteration of PS IIpropertieswithnon-photochemical excitation quenching* A. Laisk andV. Oja 1409 Downloaded from rstb.royalsocietypublishing.org



Figure 1. Measurement of changes in the active PS II pool. Figure 1. Measurement of changes in the active PS II pool.<br>Trains of flashes, 5 s apart, were given in repeated experiments<br>beginning 2, 3, 4, 5 and 6s after the activic light was replaced Figure 1. Measurement of changes in the active PS II pool.<br>Trains of flashes, 5 s apart, were given in repeated experiments<br>beginning 2, 3, 4, 5 and 6 s after the actinic light was replaced<br>by far-red light at time zero. T Trains of flashes, 5 s apart, were given in repeated experiments<br>beginning 2, 3, 4, 5 and 6 s after the actinic light was replaced<br>by far-red light at time zero. The sunflower leaf had been in<br>anaerobiosis for 20 s before. beginning 2, 3, 4, 5 and 6s after the actinic light was replaced<br>by far-red light at time zero. The sunflower leaf had been in<br>anaerobiosis for 20s before. The reference line, showing the by far-red light at time zero. The sunflower leaf had been in<br>anaerobiosis for 20s before. The reference line, showing the<br>response of photosynthesis and the  $O_2$  analyser for the change<br>in light intensity, was measured anaerobiosis for 20s before. The reference line, showing the<br>response of photosynthesis and the  $O_2$  analyser for the chang<br>in light intensity, was measured without flashes. Oxygen<br>evolution was calculated as the differe response of photosynthesis and the  $O_2$  analyser for the change<br>in light intensity, was measured without flashes. Oxygen<br>evolution was calculated as the difference between traces with<br>and without flashes in light intensity, was<br>evolution was calculat<br>and without flashes.

and without hasnes.<br>constant. Changes in leaf transpiration rate and even in<br>CO. fixation rate will change the dilution ratio of O. in constant. Changes in leaf transpiration rate and even in  $CO_2$  fixation rate will change the dilution ratio of  $O_2$  in the gas stream and cause large variations in its concentra- $CO<sub>2</sub>$  fixation rate will change the dilution ratio of  $O<sub>2</sub>$  in the gas stream and cause large variations in its concentra- $CO<sub>2</sub>$  fixation rate will change the dilution ratio of  $O<sub>2</sub>$  in<br>the gas stream and cause large variations in its concentra-<br>tion that may exceed the signal from the measured single-<br>turnouer flash the gas stream at<br>tion that may ex-<br>turnover flash. In that may exceed the signal from the measured single-<br>For multiple turnover pulse measurements the<br>nstraints are not as severe as for single-turnover flash

turnover flash.<br>For multiple turnover pulse measurements the<br>constraints are not as severe as for single-turnover flash For multiple turnover pulse measurements the<br>constraints are not as severe as for single-turnover flash<br>measurements. In leaves the PQ pool is about 20–<br>30 umole<sup>-</sup> m<sup>2</sup> (10–15 PO /PS II) Allowing for the reducconstraints are not as severe as for single-turnover flash<br>measurements. In leaves the PQ pool is about 20-<br>30 µmol e<sup>-</sup> m<sup>2</sup> (10-15 PQ/PS II). Allowing for the reduc-<br>tion of a maximum of 30% of the PO pool in one flash measurements. In leaves the PQ pool is about 20–<br>30  $\mu$ mole<sup>-</sup> m<sup>2</sup> (10–15 PQ/PS II). Allowing for the reduction of a maximum of 30% of the PQ pool in one flash,<br>the  $e^-$  transport per flash will be  $7-10 \,\mu$ mole<sup>-</sup> m<sup>-2</sup>  $30 \mu$ mol e<sup>-</sup> m<sup>2</sup> (10-15 PQ/PS II). Allowing for the reduction of a maximum of  $30\%$  of the PQ pool in one flash,  $\sigma_2$  evolution will be 2–2.5  $\mu$ mol m<sup>-2</sup>.<br> **(d)** *Requirements for the gas system*<br>
(ich sensitivity of the Q, analyser requires a yery

(d) **Requirements for the gas system**<br>The high sensitivity of the  $O_2$  analyser requires a very<br>wheckground Q, concentration of  $10-50$  umol Q, (mol (d) **Requirements for the gas system**<br>The high sensitivity of the  $O_2$  analyser requires a very<br>low background  $O_2$  concentration of 10-50 µmol  $O_2$  (mol<br> $\cos^{-1}$  This concentration is too low for normal func- $\text{gas}$ <sup>-1</sup>. This concentration is too low for normal func-Eigh sensitivity of the  $O_2$  analyser requires a very<br>concentration of  $10-50 \mu$  mol $O_2$  (mol<br>. This concentration is too low for normal func-<br>x of leaf respiration, problems with an<br>arcohiosis low background  $O_2$  concentration of 10–50 µmol  $O_2$  (mol<br>gas)<sup>-1</sup>. This concentration is too low for normal func-<br>tioning of leaf respiration, problems with anaerobiosis<br>may appear during the measurements and leaves ca  $\text{gas})^{-1}$ . This concentration is too low for normal functioning of leaf respiration, problems with anaerobiosis may appear during the measurements, and leaves can be exposed to the low  $\Omega$ , environment only for short ti tioning of leaf respiration, problems with anaerobiosis<br>may appear during the measurements, and leaves can be<br>exposed to the low  $O_2$  environment only for short time.<br>Thus a gas system that allows fast and reliable manip may appear during the measurements, and leaves can be exposed to the low  $O_2$  environment only for short time. Thus, a gas system that allows fast and reliable manipulameasurements. tion with  $O_2$  concentration is necessary for these<br>measurements.<br>Leaves were enclosed in a chamber (diameter 3.1 cm,

height  $0.3 \text{ cm}$  and exposed to a gas flow rate of Leaves were enclosed in a chamber (diameter 3.1cm,<br>height 0.3cm) and exposed to a gas flow rate of<br>0.5 mmol s<sup>-1</sup> (Fast-Est) (Oja 1983; Laisk & Oja 1998).<br>An open (flow-through) gas system was fed with pure height  $0.3 \text{ cm}$ ) and exposed to a gas flow rate of  $0.5 \text{ mmol s}^{-1}$  (Fast-Est) (Oja 1983; Laisk & Oja 1998).<br>An open (flow-through) gas system was fed with pure N and O from pressure cylinders and the necessary 0.5 mmol s<sup>-1</sup> (Fast-Est) (Oja 1983; Laisk & Oja 1998).<br>An open (flow-through) gas system was fed with pure  $N_2$  and  $O_2$  from pressure cylinders and the necessary<br>O<sub>2</sub> concentration was mixed using calibrated capillary An open (flow-through) gas system was fed with pure  $N_2$  and  $O_2$  from pressure cylinders and the necessary  $O_2$  concentration was mixed using calibrated capillary  $N_2$  and  $O_2$  from pressure cylinders and the necessary<br> $O_2$  concentration was mixed using calibrated capillary<br>flow meters. In  $N_2$  the background  $O_2$  concentration<br>was about 10–50 ppm (dependent on the cylinder of  $O_2$  concentration was mixed using calibrated capillary<br>flow meters. In  $N_2$  the background  $O_2$  concentration<br>was about 10-50 ppm (dependent on the cylinder of<br>technical grade N ) and this was used as a background flow meters. In  $N_2$  the background  $O_2$  concentration<br>was about 10-50 ppm (dependent on the cylinder of<br>technical grade  $N_2$ ) and this was used as a background<br>for single turnover flash  $O$  evolution measurements. A was about 10-50 ppm (dependent on the cylinder of measure  $O_2$  evolution from the first flash on the drifting<br>technical grade  $N_2$ ) and this was used as a background reference after 2 s from the moment of turning the ac technical grade  $N_2$ ) and this was used as a background multiple turnover pulse measurements proved to be background  $O_2$  concentration of 0.4% used in some<br>multiple turnover pulse measurements proved to be<br>adequate for stable PS II e<sup>-</sup> transport and  $q_N$ , but still<br>low enough to maintain sufficient sensitivity of the O multiple turnover pulse measurements proved to be<br>adequate for stable PS II e<sup>-</sup> transport and  $q_N$ , but still<br>low enough to maintain sufficient sensitivity of the O<sub>2</sub><br>analyser When the very low O<sub>2</sub> background was used adequate for stable PS II e<sup>-</sup> transport and  $q_N$ , but still<br>low enough to maintain sufficient sensitivity of the O<sub>2</sub><br>analyser. When the very low O<sub>2</sub> background was used,<br>the coutine of the experiments was arranged so t low enough to maintain sufficient sensitivity of the  $O_2$ <br>analyser. When the very low  $O_2$  background was used,<br>the routine of the experiments was arranged so that



lines, calculated from a model for a leaf with 10% light<br>transmission.<br>leaves were in anaerobiosis for no longer than one Figure 2. Flash–dosage curves for a sunflower leaf<br>preconditioned 30 min at an absorbed quantum flux Figure 2. Flash-dosage curves for a sunflower leaf<br>preconditioned 30 min at an absorbed quantum flux<br>density (PAD) of 30.3 µmol m<sup>-2</sup> s<sup>-1</sup> (closed squares) and<br>1700 µmol m<sup>-2</sup> s<sup>-1</sup> (open squares). Triangles indicate preconditioned 30 min at an absorbed quantum flux<br>density (PAD) of 30.3 µmol  $m^{-2} s^{-1}$  (closed squares) and<br>1700 µmol  $m^{-2} s^{-1}$  (open squares). Triangles indicate<br>measurements after reconditioning at the low PAD. Thir  $1700 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> (open squares). Triangles indicate measurements after reconditioning at the low PAD. Thin 1700  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (open squares). Triangles indicate<br>measurements after reconditioning at the low PAD. Thin<br>lines, exponents calculated for an optically thin object. Thick<br>lines, calculated from a model for a leaf w measurements after reconditioning at the low PAD. Thin<br>lines, exponents calculated for an optically thin object. Thines, calculated from a model for a leaf with 10% light<br>transmission transmission.

minute.

### **3. RESULTS AND CONCLUSIONS**

# **(a)** *A routine for the measurement of oxygen yield from single turnover flashes*<br>*from single turnover flashes*<br>*from single turnover flashes* (a) A routine for the measurement of oxygen yield<br>from single turnover flashes<br>A computer-operated routine was used in these<br>examements. Leaves were preconditioned at different

the e<sup>-</sup> transport per flash will be  $7-10 \mu$ mole<sup>-</sup> m<sup>-2</sup> and PADs in 2% O<sub>2</sub> and 330  $\mu$ molCO<sub>2</sub> mol<sup>-1</sup> to induce the **Solution Symbol Symbol Example 1.5 A**<br>
measurements. Leaves were preconditioned at different<br>
PADs in 2% Q, and 330 umol CQ, mol<sup>-1</sup> to induce the A computer-operated routine was used in these<br>measurements. Leaves were preconditioned at different<br>PADs in 2%  $O_2$  and 330 µmol  $CO_2$  mol<sup>-1</sup> to induce the<br>necessary  $a_r$ . PAD and the duration of the precondimeasurements. Leaves were preconditioned at different PADs in 2%  $O_2$  and 330  $\mu$ mol CO<sub>2</sub> mol<sup>-1</sup> to induce the necessary  $q_N$ . PAD and the duration of the precondi-PADs in 2%  $O_2$  and 330 µmol  $CO_2$  mol<sup>-1</sup> to induce the necessary  $q_N$ . PAD and the duration of the preconditioning determined whether  $q_N$  was mostly  $q_E$  or rever-<br>sible *q* type. To start, the flash measurements. O necessary  $q_N$ . PAD and the duration of the preconditioning determined whether  $q_N$  was mostly  $q_E$  or reversible  $q_I$  type. To start the flash measurements,  $O_2$  concentration was decreased to  $10-50$  umol mol<sup>-1</sup> and t tioning determined whether  $q_N$  was mostly  $q_E$  or reversible  $q_I$  type. To start the flash measurements,  $O_2$  concentration was decreased to 10-50 µmol mol<sup>-1</sup> and the following routine was applied: white actinic light sible  $q_1$  type. To start the flash measurements,  $O_2$  concentration was decreased to  $10-50 \,\mu\text{mol/mol}^{-1}$  and the following routine was applied: white actinic light was concentration was decreased to 10–50 µmol mol<sup>-1</sup> and the<br>following routine was applied: white actinic light was<br>replaced by FR light of 270 µmol m<sup>-2</sup>s<sup>-1</sup> (incident)<br>containing about 15 µmol m<sup>-2</sup>s<sup>-1</sup> of PS II light following routine was applied: white actinic light was<br>replaced by FR light of  $270 \mu \text{mol m}^{-2} \text{s}^{-1}$  (incident)<br>containing about  $15 \mu \text{mol m}^{-2} \text{s}^{-1}$  of PS II light<br>(absorbed: background O evolution under FR was the replaced by FR light of  $270 \mu \text{mol m}^{-2} \text{s}^{-1}$  (incident)<br>containing about  $15 \mu \text{mol m}^{-2} \text{s}^{-1}$  of PS II light<br>(absorbed; background O<sub>2</sub> evolution under FR was the<br>same as under  $30 \mu \text{mol m}^{-2} \text{s}^{-1}$  of white light w containing about  $15 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> of PS II light<br>(absorbed; background O<sub>2</sub> evolution under FR was the<br>same as under  $30 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> of white light, which<br>equals to  $15 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> PS II light) FR light was (absorbed; background  $O_2$  evolution under FR was the same as under 30  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> of white light, which equals to 15  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PS II light). FR light was necessary to completely oxidize PO before and bet same as under  $30 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> of white light, which<br>equals to 15  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> PS II light). FR light was neces-<br>sary to completely oxidize PQ before and between flashes<br>and low PS II light was necessary to mix equals to 15  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> PS II light). FR light was necessary to completely oxidize PQ before and between flashes<br>and low PS II light was necessary to mix s-states. Either<br>one single flash or trains of flashes. 5. sary to completely oxidize  $PQ$  before and between flashes and low  $PS$  II light was necessary to mix s-states. Either one single flash or trains of flashes, 5 s apart, were given in repeated experiments beginning  $2, 3, 4$ and low PS II light was necessary to mix s-states. Either<br>one single flash or trains of flashes, 5 s apart, were given<br>in repeated experiments beginning 2, 3, 4, 5 and 6 s after<br>the actinic light was turned off (shown with one single flash or trains of flashes, 5 s apart, were given<br>in repeated experiments beginning 2, 3, 4, 5 and 6 s after<br>the actinic light was turned off (shown with different<br>lines in figure 1: the reference line was measu the actinic light was turned off (shown with different lines in figure 1; the reference line was measured applying the actinic light was turned off (shown with different<br>lines in figure 1; the reference line was measured applying<br>the same routine without flashes). The difference between<br>the recordings with and without flashes was taken lines in figure 1; the reference line was measured applying<br>the same routine without flashes). The difference between<br>the recordings with and without flashes was taken as  $O_2$ <br>evolution from the flash. This way it was po the same routine without flashes). The difference between<br>the recordings with and without flashes was taken as  $O_2$ <br>evolution from the flash. This way it was possible to<br>measure  $O_2$  evolution from the first flash on th the recordings with and without flashes was taken as  $O_2$ <br>evolution from the flash. This way it was possible to<br>measure  $O_2$  evolution from the first flash on the drifting<br>reference after 2s from the moment of turning t evolution from the flash. This way it was possible to<br>measure  $O_2$  evolution from the first flash on the drifting<br>reference after 2 s from the moment of turning the actinic<br>light off and after every second further on Sta measure  $O_2$  evolution from the first flash on the drifting reference after 2 s from the moment of turning the actinic<br>light off and after every second further on. Standard<br>deviation of the measured  $O_2$  evolution from one flash<br>was less than  $\pm 1\%$ . Such a measurement routine light off and after every second further on. Standard deviation of the measured  $O_2$  evolution from one flash was less than  $\pm 1\%$ . Such a measurement routine allowed us to search for changes in the flash  $O$ , yield du deviation of the measured  $O_2$  evolution from one flash was less than  $\pm 1\%$ . Such a measurement routine allowed us to search for changes in the flash  $O_2$  yield during the post-illumination period with time resolutio was less than  $\pm 1\%$ . Such a measurement routine allowed<br>us to search for changes in the flash  $O_2$  yield during the<br>post-illumination period with time resolution of 1 s begin-<br>ning from 2 s after the actinic light was us to search for changes in the flash  $O_2$  yield during the post-illumination period with time resolution of 1s beginning from 2s after the actinic light was replaced by the FR light. post-illumination period with time resolution of 1s begin-

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# **(b)** *Quantum dosage response of oxygen yield from a single turnover £ash without and with* a single turnover flash without and with<br>non-photochemical quenching a single turnover flash without and with<br>
non-photochemical quenching<br>
In these experiments the phase-shifted flash trains<br>
nown in figure 1 were not applied but one flash was given

**non-photochemical quenching**<br>In these experiments the phase-shifted flash trains<br>shown in figure 1 were not applied but one flash was given<br>4.6 after actinic light was replaced by ER. Elash intensity In these experiments the phase-shifted flash trains<br>shown in figure 1 were not applied but one flash was given<br>4 s after actinic light was replaced by FR. Flash intensity<br>was changed to obtain flash-dosage curves of  $\Omega$ , shown in figure 1 were not applied but one flash was given<br>4s after actinic light was replaced by FR. Flash intensity<br>was changed to obtain flash-dosage curves of  $O_2$  evolu-<br>tion (figure 2) While the flash-dosage curve 4s after actinic light was replaced by FR. Flash intensity<br>was changed to obtain flash-dosage curves of  $O_2$  evolu-<br>tion (figure 2). While the flash-dosage curve for an opti-<br>cally thin layer is exponential, the response was changed to obtain flash-dosage curves of  $O_2$  evolution (figure 2). While the flash-dosage curve for an optically thin layer is exponential, the response of the whole leaf is a complex function a sum of exponents wit tion (figure 2). While the flash-dosage curve for an optically thin layer is exponential, the response of the whole<br>leaf is a complex function, a sum of exponents with<br>different constants. The flash-dosage curves saturate cally thin layer is exponential, the response of the whole<br>leaf is a complex function, a sum of exponents with<br>different constants. The flash-dosage curves saturate<br>more slowly than single exponents (thin lines) a result o leaf is a complex function, a sum of exponents with<br>different constants. The flash-dosage curves saturate<br>more slowly than single exponents (thin lines), a result of<br>ontical thickness of the leaf Thicker lines that fit the different constants. The flash-dosage curves saturate<br>more slowly than single exponents (thin lines), a result of<br>optical thickness of the leaf. Thicker lines that fit the data points were calculated from a computer model that optical thickness of the leaf. Thicker lines that fit the data<br>points were calculated from a computer model that<br>considered exponential attenuation of light in the leaf,<br>assuming that  $10\%$  light was transmitted through t points were calculated from a computer model that<br>considered exponential attenuation of light in the leaf,<br>assuming that  $10\%$  light was transmitted through the<br>leaf. The maximum flash of  $95$  umolquantam<sup>-2</sup> considered exponential attenuation of light in the leaf,<br>assuming that  $10\%$  light was transmitted through the<br>leaf. The maximum flash of 95 µmol quanta m<sup>-2</sup><br>(absorbed) from the  $12 \text{ uF}$  canacitor was powerful enough assuming that 10% light was transmitted through the leaf. The maximum flash of 95  $\mu$ mol quanta m<sup>-2</sup>  $\bullet$  to saturate the response.<br>The experiment was carried out with two precon-(absorbed) from the  $12 \mu$ F capacitor was powerful enough

to saturate the response.<br>The experiment was carried out with two precon-<br>ditioning PADs: 26 and 1700 µmol quanta m<sup>-2</sup> s<sup>-1</sup> and<br>exposures of 30 min. The flash Q, evolution saturated at exposures of 30 min. The flash  $O_2$  evolution saturated at ditioning PADs: 26 and 1700 µmol quanta  $m^{-2} s^{-1}$  and<br>exposures of 30 min. The flash O<sub>2</sub> evolution saturated at<br>2.2 and 1.6 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> in the low- and high-light<br>conditioned states, respectively. For the high exposures of 30 min. The flash  $O_2$  evolution saturated at 2.2 and 1.6 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> in the low- and high-light conditioned states, respectively. For the high  $q_N$  state induced by a 30 min exposure to the high PA 2.2 and 1.6  $\mu$ mole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> in the low- and high-light<br>conditioned states, respectively. For the high  $q_N$  state<br>induced by a 30 min exposure to the high PAD the<br>number of active PS II centres decreased from 2.2 to conditioned states, respectively. For the high  $q_N$  state<br>induced by a 30 min exposure to the high PAD the<br>number of active PS II centres decreased from 2.2 to<br>1.6 µmol PS II m<sup>-2</sup> or to 72.7% from the initial state at  $Q$ induced by a 30 min exposure to the high PAD the<br>number of active PS II centres decreased from 2.2 to<br>1.6 µmol PS II m<sup>-2</sup> or to 72.7% from the initial state at<br>the low PAD. However, the initial slope of the flashnumber of active PS II centres decreased from 2.2 to 1.6 µmol PS II m<sup>-2</sup> or to 72.7% from the initial state at the low PAD. However, the initial slope of the flash—dosage curve (flash quantum yield in mol e<sup>-</sup> per mol ph the low PAD. However, the initial slope of the flash-<br>dosage curve (flash quantum yield in mol  $e^-$  per mol<br>photons) decreased from 0.35 to 0.19 or to 53% of the<br>low-light adapted value. This shows that under the dosage curve (flash quantum yield in mol  $e^-$  per mol<br>photons) decreased from 0.35 to 0.19 or to 53% of the<br>low-light adapted value. This shows that under the<br>preconditioning routine the number of active PS II photons) decreased from 0.35 to 0.19 or to 53% of the low-light adapted value. This shows that under the preconditioning routine the number of active PS II low-light adapted value. This shows that under the preconditioning routine the number of active PS II centres decreased in response to the induced  $q_N$ , but, additionally antenna efficiency also decreased in each preconditioning routine the number of active PS II<br>centres decreased in response to the induced  $q_N$ , but,<br>additionally, antenna efficiency also decreased in each<br>PS II centre that remained active This state of reduced centres decreased in response to the induced  $q_N$ , but,<br>additionally, antenna efficiency also decreased in each decreased to  $1 \mu \text{mole}^{-m^{-2}}$  and increased very slowly<br>PS II centre that remained active. This state of redu additionally, antenna efficiency also decreased in each<br>PS II centre that remained active. This state of reduced<br>PS II number and lower antenna efficiency was mostly<br>reversible as seen from the curve measured after re-PS II centre that remained active. This state of reduced<br>PS II number and lower antenna efficiency was mostly<br>reversible, as seen from the curve measured after re-<br>adaptation to the low PAD for 30 min at the end of the PS II number and lower antenna efficiency was mostly<br>reversible, as seen from the curve measured after re-<br>adaptation to the low PAD for 30 min at the end of the<br>experiment (figure 2 triangles) reversible, as seen from the curve measured after re-<br>adaptation to the low PAD for 30 min at the end of the<br>experiment (figure 2, triangles).

# **(c)** *Changes in PS II accompanying energy-dependent and reversible inhibitory non-photochemical quenching* **and reversible inhibitory non-photochemical**<br> **quenching**<br>
Though the  $q_N$  induced by the 30 min exposure<br>
verted almost completely within 30 min at the low

**reverted** almost completely within 30 min exposure<br>reverted almost completely within 30 min at the low repay the relaxation kinetics were two phase suggesting Though the  $q_N$  induced by the 30 min exposure<br>reverted almost completely within 30 min at the low<br>PAD, the relaxation kinetics were two phase, suggesting<br>that two different processes were involved. In order to see reverted almost completely within 30 min at the low<br>PAD, the relaxation kinetics were two phase, suggesting<br>that two different processes were involved. In order to see<br>whether the number of active PS II was reduced during PAD, the relaxation kinetics were two phase, suggesting<br>that two different processes were involved. In order to see<br>whether the number of active PS II was reduced during<br>the faster or the slower phase of  $g_{\alpha}$ , the abov that two different processes were involved. In order to see<br>whether the number of active PS II was reduced during<br>the faster or the slower phase of  $q_N$ , the above experi-<br>ments were repeated but the time of preconditioni whether the number of active PS II was reduced during<br>the faster or the slower phase of  $q_N$ , the above experi-<br>ments were repeated but the time of preconditioning at<br>PAD of 1700 umol quanta m<sup>-2</sup>s<sup>-1</sup> was reduced to 1 mi the faster or the slower phase of  $q_N$ , the above experiments were repeated but the time of preconditioning at PAD of 1700 µmol quanta m<sup>-2</sup>s<sup>-1</sup> was reduced to 1 min.<br>Such a short exposure induced only a, type (photoprot ments were repeated but the time of preconditioning at PAD of 1700  $\mu$ mol quanta m<sup>-2</sup> s<sup>-1</sup> was reduced to 1 min.<br>Such a short exposure induced only  $q_E$  type (photoprotec-<br>tive) quenching that reversed completely withi PAD of 1700 µmol quanta  $m^{-2} s^{-1}$  was reduced to 1 min.<br>Such a short exposure induced only  $q_E$  type (photoprotective) quenching that reversed completely within 5 min.<br>The experiment was carried out according to the rout Such a short exposure induced only  $q_E$  type (photoprotective) quenching that reversed completely within 5 min.<br>The experiment was carried out according to the routine of figure 1, preconditioning the leaf at PADs of The experiment was carried out according to the routine<br>of figure 1, preconditioning the leaf at PADs of<br>51 µmol m<sup>-2</sup>s<sup>-1</sup> for 30 min and of 1700 µmol m<sup>-2</sup>s<sup>-1</sup><br>either for 1 min or 30 min. The low-light preconditioned of figure 1, preconditioning the leaf at PADs of<br>51  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> for 30 min and of 1700  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>either for 1 min or 30 min. The low-light preconditioned<br>leaf showed a constant O, vield of 1.3 umol e<sup>-</sup> m<sup></sup> 51 µmol m<sup>-2</sup>s<sup>-1</sup> for 30 min and of 1700 µmol m<sup>-2</sup><br>either for 1 min or 30 min. The low-light precondition<br>leaf showed a constant O<sub>2</sub> yield of 1.3 µmol e<sup>-</sup> m<sup>-2</sup> fr<br>repeated flashes (figure 3, open squares). After the leaf showed a constant  $O_2$  yield of 1.3 µmol  $e^-$  m<sup>-2</sup> from either for 1 min or 30 min. The low-light preconditioned<br>leaf showed a constant  $O_2$  yield of 1.3 µmol  $e^-$  m<sup>-2</sup> from<br>repeated flashes (figure 3, open squares). After the 1 min<br>conditioning at the high PAD the flash yie leaf showed a constant  $O_2$  yield of 1.3  $\mu$ mol  $e^{-}$  m<sup>-2</sup> from<br>repeated flashes (figure 3, open squares). After the 1 min<br>conditioning at the high PAD the flash yield increased<br>rapidly and approached the low-light pre repeated flashes (figure 3, open squares). After the 1 min<br>conditioning at the high PAD the flash yield increased<br>rapidly and approached the low-light preconditioned<br>level within  $10s$  (figure 3, open triangles). After the conditioning at the high PAD the flash yield increased<br>rapidly and approached the low-light preconditioned<br>level within 10 s (figure 3, open triangles). After the longer<br>exposure  $(30 \text{ min})$  to the high PAD the flash O, yi rapidly and approached the low-light preconditioned<br>level within 10 s (figure 3, open triangles). After the longer<br>exposure  $(30 \text{ min})$  to the high PAD the flash  $O_2$  yield had exposure  $(30 \text{ min})$  to the high PAD the flash  $O_2$  yield had<br>*Phil. Trans. R. Soc. Lond.* B  $(2000)$ 



Figure 3. Changes in the pool of active PS II during the Figure 3. Changes in the pool of active PS II during the relaxation of  $q_E$ . A sunflower leaf was exposed at low and high absorbed quantum flux density (PAD) and the flash Figure 3. Changes in the pool of active PS II during the<br>relaxation of  $q_E$ . A sunflower leaf was exposed at low and<br>high absorbed quantum flux density (PAD) and the flash<br>O<sub>r</sub> yield was measured under far-red light in re relaxation of  $q_E$ . A sunflower leaf was exposed at low and<br>high absorbed quantum flux density (PAD) and the flash<br> $O_2$  yield was measured under far-red light in repeated<br>phase shifted flash trains, as shown in figure 1. phase-shifted flash trains, as shown in figure 1. Open squares,  $O_2$  yield was measured under far-red light in repeated<br>phase-shifted flash trains, as shown in figure 1. Open squa<br>after an exposure at 51 µmol quanta m<sup>-2</sup> s<sup>-1</sup> for 30 min.<br>Open triangles, after an exposure at 1700 µm phase-shifted flash trains, as shown in figure 1. Open square<br>after an exposure at 51 µmol quanta  $m^{-2} s^{-1}$  for 30 min.<br>Open triangles, after an exposure at  $1700$  µmol  $m^{-2} s^{-1}$ <br>for 1 min. Closed triangles, after an ex after an exposure at 51 µmol quanta m<sup>-2</sup>s<sup>-1</sup> for 30 min.<br>Open triangles, after an exposure at 1700 µmol m<sup>-2</sup>s<sup>-1</sup><br>for 1 min. Closed triangles, after an exposure at 1700 µmol<br>quanta m<sup>-2</sup>s<sup>-1</sup> for 30 min Open triangles, after an expc<br>for 1 min. Closed triangles, a<br>quanta m<sup>-2</sup> s<sup>-1</sup> for 30 min.

quanta m<sup>-2</sup>s<sup>-1</sup> for 30 min.<br>decreased to 1 µmol e<sup>-</sup> m<sup>-2</sup> and increased very slowly<br>(figure 3 closed triangles). When the leaf was redecreased to  $1 \mu \text{mol} \text{e}^{-m^{-2}}$  and increased very slowly (figure 3, closed triangles). When the leaf was re-<br>conditioned at the low PAD for 30 min again, the flash decreased to  $1 \mu \text{mol} \text{e}^{-} \text{m}^{-2}$  and increased very slowly<br>(figure 3, closed triangles). When the leaf was re-<br>conditioned at the low PAD for 30 min again, the flash<br>wield increased to almost the initial level (not (figure 3, closed triangles). When the leaf was reconditioned at the low PAD for 30 min again, the flash yield increased to almost the initial level (not shown).<br>After hrief or prolonged exposure to bigh PAD  $\overline{F}$  was conditioned at the low PAD for 30 min again, the flash<br>yield increased to almost the initial level (not shown).<br>After brief or prolonged exposure to high PAD,  $F_m$  was<br>quenched to 40% of its initial value. During the firs After brief or prolonged exposure to high PAD,  $F_m$  was quenched to 40% of its initial value. During the first 10 s under FR there was very little change in  $F_m$ , which showed that the fast post-illumination relaxation of quenched to 40% of its initial value. During the first 10s<br>under FR there was very little change in  $F_{\text{m}}$ , which<br>showed that the fast post-illumination relaxation of the<br>PS II inhibition was not related to the  $q_{\text{E$ under FR there was very little change in  $F_{\text{m}}$ , which showed that the fast post-illumination relaxation of the<br>**PS II** inhibition was not related to the  $q_E$  quenching of<br> $F_m$ . Currently it is not known whether this very rapidly<br>reversible downrequistion of **PS** II activity PS II inhibition was not related to the  $q_E$  quenching of  $F_m$ . Currently it is not known whether this very rapidly reversible downregulation of PS II activity was a real regulatory event or it reflected the speed of plas  $F_m$ . Currently it is not known whether this very rapidly<br>reversible downregulation of PS II activity was a real<br>regulatory event or it reflected the speed of plastoquinol<br>oxidation under FR during the first 10s after the reversible downregulation of PS II activity was a real<br>regulatory event or it reflected the speed of plastoquinol<br>oxidation under FR during the first 10s after the high regulatory event or it reflected the speed of plastoquinol oxidation under FR during the first 10s after the high PAD was turned off. However, at the same  $F_m$  quenching, the short exposure to high light did not induce ch oxidation under FR during the first 10s after the high PAD was turned off. However, at the same  $F_m$  quenching, the short exposure to high light did not induce changes in the number of active PS II while the long exposure PAD was turned off. However, at the same  $F_m$  quenching,<br>the short exposure to high light did not induce changes in<br>the number of active PS II while the long exposure<br>induced a decrease of about  $25\%$  which was reversed the short exposure to high light did not induce changes in<br>the number of active PS II while the long exposure<br>induced a decrease of about 25%, which was reversed<br>within 30 min under low PAD the number of active PS II v<br>induced a decrease of about 25<br>within 30 min under low PAD.<br>These experiments showed duced a decrease of about 25%, which was reversed<br>thin 30 min under low PAD.<br>These experiments showed that no changes in the<br>mber of active PS II centres occurred that could be

within 30 min under low PAD.<br>These experiments showed that no changes in the<br>number of active PS II centres occurred that could be directly related to the rapidly reversible,  $q_E$  type nonnumber of active PS II centres occurred that could be directly related to the rapidly reversible,  $q_E$  type non-<br>photochemical quenching, while the longer exposure at the birsh PAD induced a more slowly reversible compodirectly related to the rapidly reversible,  $q_E$  type non-<br>photochemical quenching, while the longer exposure at<br>the high PAD induced a more slowly reversible compo-<br>nent of  $q_U$  and a parallel decrease in the number of a photochemical quenching, while the longer exposure at<br>the high PAD induced a more slowly reversible compo-<br>nent of  $q_N$  and a parallel decrease in the number of active<br>PS II centres by about  $25\%$ . The fast increase of t the high PAD induced a more slowly reversible component of  $q_N$  and a parallel decrease in the number of active PS II centres by about 25%. The fast increase of the active PS II pool after the short exposure to the high nent of  $q_N$  and a parallel decrease in the number of active PS II centres by about 25%. The fast increase of the active PS II pool after the short exposure to the high PAD shows that the activity of PS II is a dynamic pa PS II centres by about  $25\%$ . The fast increase of the active PS II pool after the short exposure to the high<br>PAD shows that the activity of PS II is a dynamic parameter that may change during photosynthesis, but these<br>changes are not directly related to the a<sub>ct</sub>ure quenching PAD shows that the activity of PS II is a dynamic para-<br>meter that may change during photosynthesis, but these<br>changes are not directly related to the  $q_E$ -type quenching<br>of  $F$ of  $F_{\text{m}}$ .

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turnover flashes with  $O_2$  yields from saturating and<br>limiting multiple turnover pulses. Oxygen yields from a Figure 4. Comparison of  $O_2$  yields from saturating single<br>turnover flashes with  $O_2$  yields from saturating and<br>limiting multiple turnover pulses. Oxygen yields from a<br>saturating single turnover flash, from an 8.6 ms p turnover flashes with  $O_2$  yields from saturating and<br>limiting multiple turnover pulses. Oxygen yields from a<br>saturating single turnover flash, from an 8.6 ms pulse of<br> $12.750$  umol quanta m<sup>-2</sup>s<sup>-1</sup> and a 38 6ms pulse o limiting multiple turnover pulses. Oxygen yields from<br>saturating single turnover flash, from an 8.6 ms puls<br>12 750 µmol quanta m<sup>-2</sup> s<sup>-1</sup> and a 38.6ms pulse of<br>850 µmol quanta m<sup>-2</sup> s<sup>-1</sup> were measured after the le saturating single turnover flash, from an 8.6 ms pulse of<br>12 750 µmol quanta m<sup>-2</sup> s<sup>-1</sup> and a 38.6ms pulse of<br>850 µmol quanta m<sup>-2</sup> s<sup>-1</sup> were measured after the leaf was<br>preconditioned at 37 µmol quanta m<sup>-2</sup> s<sup>-1</sup> for 12 750 µmol quanta m<sup>-2</sup>s<sup>-1</sup> and a 38.6ms pulse of<br>850 µmol quanta m<sup>-2</sup>s<sup>-1</sup> were measured after the leaf was<br>preconditioned at 37 µmol quanta m<sup>-2</sup>s<sup>-1</sup> for 1h (low 1) at<br>1700 µmol quanta m<sup>-2</sup>s<sup>-1</sup> for 1min (1 min 170 preconditioned at 37  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup> for 1h (low 1) at 1700  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup> for 1min (1 min 1700), at the sam absorbed quantum flux density (PAD) for 1h (1 h 1700) and For the leaf was<br>quanta  $m^{-2}s^{-1}$  for 1h (low 1) at<br>for 1 min (1 min 1700), at the same<br>poity (PAD) for 1h (1 h 1700) and preconditioned at 37 µmol quanta  $m^{-2} s^{-1}$  for 1h (low 1) at<br>1700 µmol quanta  $m^{-2} s^{-1}$  for 1 min (1 min 1700), at the same<br>absorbed quantum flux density (PAD) for 1h (1 h 1700) and<br>again at 37 µmol quanta  $m^{-2} s^{-1}$  fo 1700 µmol quanta m<sup>-2</sup> s<sup>-1</sup> for 1min (1 min 1700), at the sar<br>absorbed quantum flux density (PAD) for 1h (1 h 1700) and<br>again at 37 µmol quanta m<sup>-2</sup> s<sup>-1</sup> for 15 min (low 2). The<br>initial slope and maximum rate *V* (plat absorbed quantum flux density (PAD) for 1h (1h 1700) and<br>again at 37 µmol quanta  $m^{-2} s^{-1}$  for 15 min (low 2). The<br>initial slope and maximum rate  $V_m$  (plateau) of the hyperbolic<br>PS II light response curve were calculate again at 37 µmol quanta m<sup>-2</sup>s<sup>-1</sup> for 15 min (low 2). The<br>initial slope and maximum rate  $V_{\rm m}$  (plateau) of the hyperbolic<br>**PS II** light-response curve were calculated from the pulse O<sub>2</sub><br>yields and plotted relative t PS II light-response curve were calculated from the pulse  $O_2$  yields and plotted relative to the same values measured after the initial conditioning at the low light.

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# **(d)** *Comparison of single turnover £ashes and multiple turnover pulses* (d) Comparison of single turnover flashes<br>and multiple turnover pulses<br>Saturating single turnover flashes detect the number of<br>evolving PS II centres. Oxygen vield from high inten-

**and multiple turnover pulses**<br>Saturating single turnover flashes detect the number of<br> $O_2$  evolving PS II centres. Oxygen yield from high inten-<br>sity (saturating) multiple turnover pulses reflects the Saturating single turnover flashes detect the number of  $O_2$  evolving PS II centres. Oxygen yield from high intensity (saturating) multiple turnover pulses reflects the maximum  $e^-$  transport rate through active PS II ce  $O_2$  evolving PS II centres. Oxygen yield from high intensity (saturating) multiple turnover pulses reflects the maximum  $e^-$  transport rate through active PS II centres, while  $O$  evolution from low intensity pulses ref sity (saturating) multiple turnover pulses reflects the maximum  $e^-$  transport rate through active PS II centres, while  $O_2$  evolution from low intensity pulses reflects the intrinsic quantum vield of PS II. As described maximum  $e^-$  transport rate through active PS II centres, decrease of the low-light quantum yield, but only a less<br>while  $O_2$  evolution from low intensity pulses reflects the than 10% decrease in the maximum PS II turnov  $e^-$  transported during the pulse did not exceed sure under high PAD, causing a  $ca. 30\%$  decrease of the was pre-oxidized i<br>e<sup>-</sup> transported<br>8 µmol e<sup>-</sup> m<sup>-2</sup>, i.e  $-2$ ; ed in these experiments and the number of<br>ed during the pulse did not exceed<br>i, i.e. four e<sup>-</sup> per PS II. For better compari-<br>ements with bigh- and low-intensity pulses  $e^-$  transported during the pulse did not exceed  $8 \mu$ mol $e^-$  m<sup>-2</sup>, i.e. four  $e^-$  per PS II. For better comparison of measurements with high- and low-intensity pulses the number of  $e^-$  transported in comparable experim  $8 \mu$ mol e<sup>-</sup> m<sup>-2</sup>, i.e. four e<sup>-</sup> per PS II. For better comparison of measurements with high- and low-intensity pulses the number of e<sup>-</sup> transported in comparable experiments was made approximately equal by changing th son of measurements with high- and low-intensity pulses of active PS II, but the latter effect was not related to  $F_m$  the number of  $e^-$  transported in comparable experiments quenching and reversed within 10 s.<br>was made the number of  $e^-$  transported in comparable experiments was made approximately equal by changing the duration<br>of the pulse. It was therefore ensured that the acceptor<br>side limitation due to PQ reduction did not exceed 15%<br>on average and was equal in comparable experiments of the pulse. It was therefore ensured that the accept<br>side limitation due to PQ reduction did not exceed 15<br>on average and was equal in comparable experiments.<br>Comparable measurements applying a saturating flas le limitation due to PQ reduction did not exceed 15%<br>average and was equal in comparable experiments.<br>Comparable measurements applying a saturating flash,<br>high-intensity, multiple, turnover, pulse, and a low-

on average and was equal in comparable experiments.<br>Comparable measurements applying a saturating flash,<br>a high-intensity multiple turnover pulse and a low-Comparable measurements applying a saturating flash,<br>a high-intensity multiple turnover pulse and a low-<br>intensity multiple turnover pulse were carried out 5 s<br>after the white actinic light was replaced by FR. The a high-intensity multiple turnover pulse and a low-<br>intensity multiple turnover pulse were carried out 5 s<br>after the white actinic light was replaced by FR. The<br>preceding exposure at 1700 umplementa  $m^{-2} s^{-1}$  was intensity multiple turnover pulse were carried after the white actinic light was replaced by FF<br>preceding exposure at 1700 µmol quanta m<sup>-2</sup>s<sup>-</sup><br>either l min or 60 min. Subsequent to the l min after the white actinic light was replaced by FR. The preceding exposure at 1700 µmol quanta  $m^{-2} s^{-1}$  was either 1min or 60 min. Subsequent to the 1min pre-<br>conditioning at 1700 µmol quanta  $m^{-2} s^{-1}$  O, yield from preceding exposure at 1700 µmol quanta  $m^{-2} s^{-1}$  was<br>either 1 min or 60 min. Subsequent to the 1 min pre-<br>conditioning at 1700 µmol quanta  $m^{-2} s^{-1}$ , O<sub>2</sub> yield from 15<br>flashes decreased by 9% compared with the preeither 1 min or 60 min. Subsequent to the 1 min pre-<br>conditioning at 1700 µmol quanta  $m^{-2} s^{-1}$ , O<sub>2</sub> yield from<br>flashes decreased by 9% compared with the pre-<br>conditioning at PAD of 37 µmol  $m^{-2} s^{-1}$  (figure 4). As in conditioning at 1700 µmol quanta  $m^{-2} s^{-1}$ ,  $O_2$  yield from<br>flashes decreased by 9% compared with the pre-<br>conditioning at PAD of 37 µmol  $m^{-2} s^{-1}$  (figure 4). As in<br>the experiment shown in figure 3, this small decreas flashes decreased by 9% compared with the pre-<br>conditioning at PAD of 37  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (figure 4). As in<br>the experiment shown in figure 3, this small decrease<br>detected in the active PS II pool was not related to the conditioning at PAD of 37  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> (figure 4). As in<br>the experiment shown in figure 3, this small decrease<br>detected in the active PS II pool was not related to the<br>a<sub>ctive</sub> quenching of  $F$ . Pulse O, yield from the experiment shown in figure 3, this small decrease detected in the active PS II pool was not related to the  $q_E$ -type quenching of  $F_m$ . Pulse O<sub>2</sub> yield from the high-

intensity multiple turnover pulses of  $12\,800\,\mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>(absorbed) and 8.6 ms duration decreased slightly more intensity multiple turnover pulses of  $12\,800\,\mu\text{mol}\,\text{m}^{-2}\,\text{s}^{-1}$ <br>(absorbed) and 8.6 ms duration decreased slightly more<br>than the flash  $\Omega$ , yield (by 16%) while the pulse yield (absorbed) and 8.6 ms duration decreased slightly more than the flash  $O_2$  yield (by 16%), while the pulse yield (absorbed) and 8.6 ms duration decreased slightly more<br>than the flash  $O_2$  yield (by 16%), while the pulse yield<br>from low-intensity pulses (850 µmol m<sup>-2</sup>s<sup>-1</sup>, 38.6 ms)<br>decreased by 27% after lunin exposure under than the flash  $O_2$  yield (by 16%), while the pulse yield<br>from low-intensity pulses (850 µmol m<sup>-2</sup>s<sup>-1</sup>, 38.6 ms)<br>decreased by 27% after 1min exposure under<br>1700 µmol guanta m<sup>-2</sup>s<sup>-1</sup>. The maximum pulse did not from low-intensity pulses  $(850 \,\mu\text{mol m}^{-2} \text{s}^{-1}, 38.6 \text{ ms})$ <br>decreased by 27% after 1 min exposure under<br>1700  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup>. The maximum pulse did not<br>completely saturate the **PS II** yield Knowing that the  $s^{-1}$ . T decreased by 27% after 1 min exposure under<br>1700  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup>. The maximum pulse did not<br>completely saturate the PS II yield. Knowing that the<br>PS II light-response curve is a rectangular bunerbola (see 1700 µmol quanta m<sup>-2</sup> s<sup>-1</sup>. The maximum pulse did not<br>completely saturate the PS II yield. Knowing that the<br>PS II light-response curve is a rectangular hyperbola (see<br>figure 9) the initial slope (quantum yield)  $\hat{Y}$  completely saturate the PS II yield. Knowing that the PS II light-response curve is a rectangular hyperbola (see figure 9), the initial slope (quantum yield)  $\hat{Y}$  and the PS II light-response curve is a rectangular hyperbola (see figure 9), the initial slope (quantum yield)  $\hat{T}$  and the maximum rate  $V_m$  was calculated and the relative changes of these parameters is given in figure 4. Af figure 9), the initial slope (quantum yield)  $\Upsilon$  and the maximum rate  $V_m$  was calculated and the relative changes of these parameters is given in figure 4. After the 1min exposure under the PAD of 1700 umol quanta  $m^{-2$ maximum rate  $V_{\rm m}$  was calculated and the relative character of these parameters is given in figure 4. After the lexposure under the PAD of 1700 µmol quanta m<sup>-2</sup>s<sup>-</sup>maximum PS II turnover rate decreased in proportion  $s^{-1}$  the of these parameters is given in figure 4. After the 1min<br>exposure under the PAD of 1700  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup> the<br>maximum PS II turnover rate decreased in proportion to exposure under the PAD of 1700  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup> the<br>maximum PS II turnover rate decreased in proportion to<br>the pool of active PS II, while the quantum yield of PS II<br>at low light, represented by the initial slope maximum PS II turnover rate decreased in proportion to<br>the pool of active PS II, while the quantum yield of PS II<br>at low light, represented by the initial slope of the light-<br>response curve decreased more the pool of active PS II, while the<br>at low light, represented by the in<br>response curve, decreased more.<br>Again, these experiments show low light, represented by the initial slope of the light-<br>sponse curve, decreased more.<br>Again, these experiments show that the rapidly indu-<br>and rapidly relaxing  $a_0$ -type quenching influences

response curve, decreased more.<br>Again, these experiments show that the rapidly indu-<br>cible and rapidly relaxing  $q_E$ -type quenching influences<br>neither the number of active PS II centres por decreases Again, these experiments show that the rapidly indu-<br>cible and rapidly relaxing  $q_E$ -type quenching influences<br>neither the number of active PS II centres nor decreases<br>the maximum turnover rate of the centres at saturatin cible and rapidly relaxing  $q_E$ -type quenching influences<br>neither the number of active PS II centres nor decreases<br>the maximum turnover rate of the centres at saturating<br>PAD but considerably decreases the quantum yield of neither the number of active PS II centres nor decreases<br>the maximum turnover rate of the centres at saturating<br>PAD, but considerably decreases the quantum yield of<br>PS II at low PAD the maximum turn<br>PAD, but consideraries<br>PS II at low PAD. PAD, but considerably decreases the quantum yield of PS II at low PAD.

 $m^{-2} s^{-1}$  the number of active PS II detected from the After the 60 min exposure from a PAD of  $1700 \mu$  mol After the 60 min exposure from a PAD of 1700 µmol<br>m<sup>-2</sup>s<sup>-1</sup> the number of active PS II detected from the<br>flash O<sub>2</sub> yield decreased by 20% and this downregulation<br>reversed within 15 min to 89% of the initial value (right  $m^{-2} s^{-1}$  the number of active PS II detected from the flash  $O_2$  yield decreased by 20% and this downregulation reversed within 15 min to 89% of the initial value (right-<br>most bars in figure 4) and continued to recover flash  $O_2$  yield decreased by 20% and this downregulation<br>reversed within 15 min to 89% of the initial value (right-<br>most bars in figure 4) and continued to recover. After this<br>exposure the calculated PS II maximum turno reversed within 15 min to 89% of the initial value (right-<br>most bars in figure 4) and continued to recover. After this<br>exposure, the calculated PS II maximum turnover rate<br> $V$  decreased slightly less than the number of ce most bars in figure 4) and continued to recover. After this exposure, the calculated PS II maximum turnover rate  $V_m$  decreased slightly less than the number of centres, but the difference is not considered meaningful bec exposure, the calculated PS II maximum turnover rate  $V_m$  decreased slightly less than the number of centres, but the difference is not considered meaningful because  $V_m$  was extrapolated. The initial slope (quantum yield the difference is not considered meaningful because  $V_m$ <br>was extrapolated. The initial slope (quantum yield)<br>decreased to 0.58 of the initial value. After 15 min of  $q_N$ <br>relaxation both  $V_a$  and quantum yield recovered to was extrapolated. The initial slope (quantum yield) decreased to 0.58 of the initial value. After 15 min of  $q_N$  relaxation, both  $V_m$  and quantum yield recovered to 90% of the initial value and continued to recover. The decreased to 0.58 of the initial value. After 15 min of  $q_N$  relaxation, both  $V_m$  and quantum yield recover. The results of the initial value and continued to recover. The results showed that the 1h exposure under 1700 u relaxation, both  $V_m$  and quantum yield recovered to 90%<br>of the initial value and continued to recover. The results<br>showed that the 1h exposure under 1700  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>induced a reversible photoinbibitory effect tha of the initial value and continued to recover. The results<br>showed that the 1h exposure under  $1700 \mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>induced a reversible photoinhibitory effect that decreased<br>flash yield and high- and low-intensity pulse showed that the 1h exposure under  $1700 \mu$ mol m<sup>-2</sup>s<sup>-1</sup><br>induced a reversible photoinhibitory effect that decreased<br>flash yield and high- and low-intensity pulse O<sub>2</sub> yield (all<br>by 10%). In parallel with the photoinhibiti induced a reversible photoinhibitory effect that decreased<br>flash yield and high- and low-intensity pulse  $O_2$  yield (all<br>by 10%). In parallel with the photoinhibition, in active flash yield and high- and low-intensity pulse  $O_2$  yield (all<br>by 10%). In parallel with the photoinhibition, in active<br>**PS II** centres the  $q_E$  quenching caused a remarkable<br>decrease of the low-light quantum yield, but o by 10%). In parallel with the photoinhibition, in active<br>PS II centres the  $q_E$  quenching caused a remarkable<br>decrease of the low-light quantum yield, but only a less<br>than 10% decrease in the maximum PS II turnover rate PS II centres the  $q_E$  quenching caused a remarkable<br>decrease of the low-light quantum yield, but only a less<br>than 10% decrease in the maximum PS II turnover rate.<br>In PS II centres that were not photoinhibited the effect decrease of the low-light quantum yield, but only a less than 10% decrease in the maximum PS II turnover rate.<br>In PS II centres that were not photoinhibited the effect of  $q_E$  quenching was similar before and after the long expo-<br>sure under high PAD, causing a ca. 30% decrease In PS II centres that were not photoinhibited the effect of  $q_E$  quenching was similar before and after the long exposure under high PAD, causing a *ca*. 30% decrease of the quantum vield and a 10% decrease of  $V$ , and th  $q_E$  quenching was similar before and after the long exposure under high PAD, causing a *ca*. 30% decrease of the quantum yield and a 10% decrease of  $V_m$  and the number of active PS II, but the latter effect was not rela sure under high PAD, causing a *ca*. 30% decrease of the quantum yield and a 10% decrease of  $V_m$  and the number of active PS II, but the latter effect was not related to  $F_m$  quenching and reversed within 10s. quantum yield and a 10% decrease of<br>of active PS II, but the latter effect v<br>quenching and reversed within 10 s.

# **(e)** *Oxygen evolution from multiple turnover pulses: dependence on pulse length* Oxygen evolution from multiple turnover pulses:<br>
dependence on pulse length<br>
In these studies the kinetics of  $O_2$  evolution and elec-<br>
no transport through PS II during the illumination with

**dependence on pulse length**<br>In these studies the kinetics of  $O_2$  evolution and electron transport through PS II during the illumination with<br>multiple turnover pulses were analysed in greater detail In these studies the kinetics of  $O_2$  evolution and electron transport through PS II during the illumination with multiple turnover pulses were analysed in greater detail.<br>As already emphasized, the short pulses were pec tron transport through PS II during the illumination with<br>multiple turnover pulses were analysed in greater detail.<br>As already emphasized, the short pulses were necessary<br>in order to avoid PO reduction and to detect accept multiple turnover pulses were analysed in greater detail.<br>As already emphasized, the short pulses were necessary<br>in order to avoid PQ reduction and to detect acceptor<br>side unlimited PS II kinetics As already emphasized, the sl<br>in order to avoid PQ reducti<br>side unlimited PS II kinetics.<br>Heing the specially designed in order to avoid PQ reduction and to detect acceptor<br>side unlimited PS II kinetics.<br>Using the specially designed shutter fitted to the KL

1500 light source, the time kinetics of  $O_2$  evolution were Using the specially designed shutter fitted to the KL<br>1500 light source, the time kinetics of  $O_2$  evolution were<br>resolved by measuring how the total  $O_2$  evolution per<br>pulse increased with increasing pulse length (figu 1500 light source, the time kinetics of  $O_2$  evolution were<br>resolved by measuring how the total  $O_2$  evolution per<br>pulse increased with increasing pulse length (figure 5).<br>The slope of the graphs obtained by increasing resolved by measuring how the total  $O_2$  evolution per<br>pulse increased with increasing pulse length (figure 5).<br>The slope of the graphs obtained by increasing pulse<br>length at a constant pulse PAD represents the  $O$ pulse increased with increasing pulse length (figure 5).<br>The slope of the graphs obtained by increasing pulse<br>length at a constant pulse PAD represents the  $O_2$ <br>evolution rate as a function of time during the pulses The slope of the graphs obtained by increasing pulse<br>length at a constant pulse PAD represents the  $O_2$ <br>evolution rate as a function of time during the pulses.



Figure 5. Total  $O_2$  evolution from a light pulse dependent<br>on the pulse length and intensity for a suppose leaf Figure 5. Total  $O_2$  evolution from a light pulse depend<br>on the pulse length and intensity for a sunflower leaf<br>pre-conditioned (a) at low light and (b) at bight Figure 5. Total  $O_2$  evolution from a light pulse depend<br>on the pulse length and intensity for a sunflower leaf<br>pre-conditioned (*a*) at low light and (*b*) at high light.<br>Pulse absorbed quantum flux densities (PADs) wer on the pulse length and intensity for a sunflower leaf<br>pre-conditioned (*a*) at low light and (*b*) at high light.<br>Pulse absorbed quantum flux densities (PADs) were<br>(downward for curves)  $13\,500, 10\,200, 6800, 3600, 172$ pre-conditioned (*a*) at low light and (*b*) at high light.<br>Pulse absorbed quantum flux densities (PADs) were<br>(downward for curves) 13 500, 10 200, 6800, 3600, 1720<br>and 880 umol  $m^{-2}e^{-1}$  Approximation with a hyperbola i Pulse absorbed quantum flux densities (PADs) were<br>(downward for curves) 13 500, 10 200, 6800, 3600, 1720<br>and 880 µmol m<sup>-2</sup> s<sup>-1</sup>. Approximation with a hyperbola is<br>shown for the unnermost curve. The slope of the dotted l (downward for curves) 13500, 10200, 6800, 3600, 1720<br>and 880  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Approximation with a hyperbola is<br>shown for the uppermost curve. The slope of the dotted line and 880 µmol m<sup>-2</sup>s<sup>-1</sup>. Approximation with a hyperbola is<br>shown for the uppermost curve. The slope of the dotted line<br>represents O<sub>2</sub> evolution rate 50 ms after the beginning of the<br>pulse (360 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup>) shown for the uppermost currepresents  $O_2$  evolution rat<br>pulse (360 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> pulse  $(360 \,\mathrm{\mu mol\,e^{-} m^{-2} s^{-1}})$ .

Experimental data points were approximated by non-Experimental data points were approximated by non-<br>rectangular hyperbolic relationships, as shown for the<br>maximum pulse PAD Clearly the slope of the byperbola Experimental data points were approximated by non-<br>rectangular hyperbolic relationships, as shown for the<br>maximum pulse PAD. Clearly, the slope of the hyperbola<br>was high at short pulse length and it decreased rapidly rectangular hyperbolic relationships, as shown for the<br>maximum pulse PAD. Clearly, the slope of the hyperbola<br>was high at short pulse length and it decreased rapidly<br>with increasing pulse length as PO became more  $\rightarrow$  maximum pulse PAD. Clearly, the slope of the hyperbola<br>  $\rightarrow$  was high at short pulse length and it decreased rapidly<br>  $\rightarrow$  with increasing pulse length as PQ became more was high at short pulse length and it decreased rapidly<br>with increasing pulse length as PQ became more<br>reduced. At pulse lengths longer than 50 ms electron<br>transport rate (temporarily) stabilized because PO reducwith increasing pulse length as PQ became more<br>reduced. At pulse lengths longer than 50 ms electron<br>transport rate (temporarily) stabilized because PQ reduc-<br>tion had reached an equilibrium steady state determined reduced. At pulse lengths longer than 50 ms electron<br>transport rate (temporarily) stabilized because PQ reduc-<br>tion had reached an equilibrium steady state determined<br>by the balance between reduction and oxidation rates transport rate (temporarily) stabilized because PQ reduction had reached an equilibrium steady state determined<br>by the balance between reduction and oxidation rates.<br>The pool of reduced PQ in this state was estimated from tion had reached an equilibrium steady state determined<br>by the balance between reduction and oxidation rates.<br>The pool of reduced PQ in this state was estimated from<br>the extrapolation of the dotted line to the ordinate ax by the balance between reduction and oxidation rates.<br>The pool of reduced  $PQ$  in this state was estimated from<br>the extrapolation of the dotted line to the ordinate axis. In the low-light adapted state this estimate was the extrapolation of the dotted line to the ordinate axis.<br>In the low-light adapted state this estimate was<br> $26 \mu \text{mol} \cdot \text{m}^{-2}$  and in the high-light adapted state it was<br> $20 \mu \text{mol} \cdot \text{m}^{-2}$ . This method of estimation In the low-light<br>  $26 \mu$ mol e<sup>-</sup> m<sup>-2</sup> and<br>  $20 \mu$ mol e<sup>-</sup> m<sup>-2</sup>. T  $-2$ ght adapted state this estimate was<br>and in the high-light adapted state it was<br>. This method of estimation of the<br>cool considered that the oxidation rate 26  $\mu$ mol e<sup>-</sup> m<sup>-2</sup> and in the high-light adapted state it was<br>20  $\mu$ mol e<sup>-</sup> m<sup>-2</sup>. This method of estimation of the<br>reduced PQ pool considered that the oxidation rate<br>saturated at very low levels of reduced PO being 20  $\mu$ mol  $e^{-}$  m<sup>-2</sup>. This method of estimation of the reduced PQ pool considered that the oxidation rate saturated at very low levels of reduced PQ, being practically independent of the pulse length. Considering that reduced PQ pool considered that the oxidation rate<br>saturated at very low levels of reduced PQ, being practi-<br>cally independent of the pulse length. Considering that

the pool of PS II in these leaves was about  $2 \mu$ mol m<sup>-2</sup>,<br>there were about ten to 13 doubly reduced PQ molecules<br>per PS II.<br>The initially high  $Q_2$  evolution rate (up to there were about ten to 13 doubly reduced PQ molecules per PS II. ere were about ten to 13 doubly reduced PQ molecules<br>r PS II.<br>The initially high  $O_2$  evolution rate (up to 00 umol m<sup>-2</sup>s<sup>-1</sup> in the low-light conditioned state)

per PS II.<br>The initially high<br>2000  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> in<br>decreased to 360 um igh  $O_2$  evolution rate (up to<br>in the low-light conditioned state)<br>pole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> with fully reduced PO The initially high  $O_2$  evolution rate (up to 2000 µmol m<sup>-2</sup>s<sup>-1</sup> in the low-light conditioned state) decreased to 360 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> with fully reduced PQ 2000 µmol m<sup>-2</sup>s<sup>-1</sup> in the low-light conditioned state)<br>decreased to 360 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> with fully reduced PQ<br>(rate calculated from the slope of the dotted line at pulse<br>length longer than 50 ms) and this rate was decreased to 360 µmole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> with fully reduced PQ<br>(rate calculated from the slope of the dotted line at pulse<br>length longer than 50 ms), and this rate was similar in the<br>low- and high-light conditioned states. Th (rate calculated from the slope of the dotted line at pulse length longer than  $50 \text{ ms}$ ), and this rate was similar in the low- and high-light conditioned states. This rate evidently length longer than 50 ms), and this rate was similar in the<br>low- and high-light conditioned states. This rate evidently<br>characterized the maximum rate of PQ oxidation by<br>cytochrome  $h$ ,  $f$  in the absence of proton gradie cytochrome  $b_6 f$  in the absence of proton gradient, a kind<br>of uncoupled electron transport rate in an intact leaf.<br>After a few seconds this rate further decreased to<br>225 umole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> evidently because a proton gra *f* light conditioned states. This rate evidently<br>the maximum rate of PQ oxidation by<br>f in the absence of proton gradient, a kind<br>electron transport rate in an intact leaf characterized the maximum rate of PQ oxidation by<br>cytochrome  $b_6 f$  in the absence of proton gradient, a kind<br>of uncoupled electron transport rate in an intact leaf.<br>After a few seconds this rate further decreased to of uncoupled electron transport rate in an intact leaf.<br>After a few seconds this rate further decreased to<br>225  $\mu$ mol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup>, evidently because a proton gradient<br>was generated Finally the rate declined to an acc After a few seconds this rate further decreased to 225 µmol  $e^-$  m<sup>-2</sup>s<sup>-1</sup>, evidently because a proton gradient was generated. Finally, the rate declined to an acceptor-<br>limited value of 120 µmol  $e^-$  m<sup>-2</sup>s<sup>-1</sup> when the 225 µmol  $e^-$  m<sup>-2</sup>s<sup>-1</sup>, evidently because a proton gradient<br>was generated. Finally, the rate declined to an acceptor-<br>limited value of 120 µmol  $e^-$  m<sup>-2</sup>s<sup>-1</sup> when the pre-<br>accumulated 3-phosphoglyceric acid (PGA) pool was generated. Finally, the rate declined to an acceptor-<br>limited value of  $120 \mu$ mole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> when the pre-<br>accumulated 3-phosphoglyceric acid (PGA) pool was<br>consumed by reduction and CO diffusion became rate limited value of  $120 \mu$ mole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> when the pre-<br>accumulated 3-phosphoglyceric acid (PGA) pool was<br>consumed by reduction and  $CO_2$  diffusion became rate<br>limiting (not shown). These rates demonstrate the  $\alpha$  accumulated 3-phosphoglyceric acid (PGA) pool was<br>consumed by reduction and  $CO_2$  diffusion became rate<br>limiting (not shown). These rates demonstrate the<br>temporal internlay between sequential rate limiting consumed by reduction and  $CO_2$  diffusion became rate<br>limiting (not shown). These rates demonstrate the<br>temporal interplay between sequential rate limiting<br>processes in photosynthetic  $e^-$  transport, the next limiting (not shown). These rates demonstrate the<br>temporal interplay between sequential rate limiting<br>processes in photosynthetic  $e^-$  transport, the next<br>becoming active after the pool of the preceding intertemporal interplay between sequential rate limiting<br>processes in photosynthetic  $e^-$  transport, the next<br>becoming active after the pool of the preceding inter-<br>mediate was consumed processes in photosynthetic  $e^-$  transport, the next<br>becoming active after the pool of the preceding inter-<br>mediate was consumed.

# **(f)** *Oxygen evolution during pulses:* **Exygen evolution during pulses:**<br>**dependence on pulse PAD**<br>rates, of Q, evolution, with c

**dependence on pulse PAD**<br>The initial rates of  $O<sub>2</sub>$  evolution with completely oxidized PQ pool were calculated from the slope of the The initial rates of  $O_2$  evolution with completely<br>oxidized PQ pool were calculated from the slope of the<br>curves after  $3 \mu$ mol e<sup>-</sup> m<sup>-2</sup> were transported. Such a<br>threshold value was used because fluorescence measureoxidized PQ pool were calculated from the slope of the<br>curves after  $3 \mu$ mole<sup>-</sup> m<sup>-2</sup> were transported. Such a<br>threshold value was used because fluorescence measure-<br>ments (below) indicated that the first one to two e<sup>-</sup> curves after  $3 \mu$ mole<sup>-</sup> m<sup>-2</sup> were transported. Such a threshold value was used because fluorescence measurements (below) indicated that the first one to two e<sup>-</sup> were transported at a higher speed than the following e<sup></sup> threshold value was used because fluorescence measure-<br>ments (below) indicated that the first one to two  $e^-$  were<br>transported at a higher speed than the following  $e^-$ ,<br>probably because the first  $e^-$  did not exchange wi transported at a higher speed than the following  $e^-$ ,<br>probably because the first  $e^-$  did not exchange with the<br>free PQ pool but stayed on the bound quinones on PS II<br>acceptor side. These  $\Omega$ , evolution rates were plott probably because the first  $e^-$  did not exchange with the<br>free PQ pool but stayed on the bound quinones on PS II<br>acceptor side. These  $O_2$  evolution rates were plotted as free PQ pool but stayed on the bound quinones on PS II acceptor side. These  $O_2$  evolution rates were plotted as light-response curves for PS II e<sup>-</sup> transport from the OEC to PQ (figure 6, open symbols). The light-respo acceptor side. These  $O_2$  evolution rates were plotted as<br>light-response curves for PS II  $e^-$  transport from the<br>OEC to PQ (figure 6, open symbols). The light-response<br>curves of PS II electron transport fit well to rect light-response curves for PS II e<sup>-</sup> transport from the OEC to PQ (figure 6, open symbols). The light-response curves of PS II electron transport fit well to rectangular hyperbols as seen from the match of the experimenta OEC to PQ (figure 6, open symbols). The light-response<br>curves of PS II electron transport fit well to rectangular<br>hyperbola, as seen from the match of the experimental<br>points of Q evolution (open symbols) with calculated curves of PS II electron transport fit well to rectangular<br>hyperbola, as seen from the match of the experimental<br>points of O<sub>2</sub> evolution (open symbols) with calculated<br>hyperbolic curves. The half-caturation PAD (K) of th hyperbola, as seen from the match of the experimental<br>points of  $O_2$  evolution (open symbols) with calculated<br>hyperbolic curves. The half-saturation PAD ( $K_m$ ) of the<br>function was 7600 umol  $m^{-2} s^{-1}$  in the low-light ad points of  $O_2$  evolution (open symbols) with calculated<br>hyperbolic curves. The half-saturation PAD ( $K_m$ ) of the<br>function was 7600 µmol m<sup>-2</sup>s<sup>-1</sup> in the low-light adapted<br>and 7400 µmol m<sup>-2</sup>s<sup>-1</sup> in the high-light adapt hyperbolic curves. The half-saturation PAD ( $K_m$ ) of the function was 7600 µmol m<sup>-2</sup> s<sup>-1</sup> in the low-light adapted and 7400 µmol m<sup>-2</sup> s<sup>-1</sup> in the high-light adapted state, showing that the maximum rate and initial slo  $s^{-1}$  in function was  $7600 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> in the low-light adapted<br>and  $7400 \mu$ mol m<sup>-2</sup>s<sup>-1</sup> in the high-light adapted state,<br>showing that the maximum rate and initial slope de-<br>creased rather proportionally. The highest exper and 7400  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> in the high-light adapted state,<br>showing that the maximum rate and initial slope de-<br>creased rather proportionally. The highest experimentally<br>available PADs were nearly twice the K and the ver showing that the maximum rate and initial slope decreased rather proportionally. The highest experimentally available PADs were nearly twice the  $K<sub>m</sub>$  and the very creased rather proportionally. The highest experimentally<br>available PADs were nearly twice the  $K_m$  and the very<br>good fit of the recorded data to rectangular hyperbolae<br>allowed us safely to extrapolate the maximum (platea available PADs were nearly twice the  $K_{\rm m}$  and the very<br>good fit of the recorded data to rectangular hyperbolae<br>allowed us safely to extrapolate the maximum (plateau)<br>values for PS II electron transport. Both, the init good fit of the recorded data to rectangular hyperbolae<br>allowed us safely to extrapolate the maximum (plateau)<br>values for PS II electron transport. Both, the initial slope<br>(intrinsic quantum yield  $\hat{Y}$ ) and the plateau allowed us safely to extrapolate the maximum (plateau) values for PS II electron transport. Both, the initial slope (intrinsic quantum yield  $Y_m$ ) and the plateau  $V_m$  of the hyperholic light-response curve of the PS II e values for PS II electron transport. Both, the initial slope<br>(intrinsic quantum yield  $\mathcal{V}_{m}$ ) and the plateau  $\mathcal{V}_{m}$  of the<br>hyperbolic light-response curve of the PS II electron<br>transport decreased when the precon (intrinsic quantum yield  $Y_m$ ) and the plateau  $V_m$  of the hyperbolic light-response curve of the PS II electron transport decreased when the preconditioning PAD was increased and  $q_m$  plus the reversible  $q_n$  increased f hyperbolic light-response curve of the PS II electron<br>transport decreased when the preconditioning PAD was<br>increased and  $q_E$  plus the reversible  $q_I$  increased from the<br>minimum to the maximum value The values of the extr transport decreased when the preconditioning PAD was<br>increased and  $q_E$  plus the reversible  $q_I$  increased from the<br>minimum to the maximum value. The values of the extra-<br>polated maximum PS II electron transport rate reac increased and  $q_E$  plus the reversible  $q_I$  increased from the<br>minimum to the maximum value. The values of the extra-<br>polated maximum PS II electron transport rate reached<br>2860 umole<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> in the low-light conditi minimum to the maxi<br>polated maximum PS<br>2860 µmol e<sup>-</sup> m<sup>-2</sup> s<sup>-1</sup><br>the leaf and decreased maximum value. The values of the extra-<br>im PS II electron transport rate reached<br> $^{-2}$ s<sup>-1</sup> in the low-light conditioned state of<br>rreased to 1450 umol m<sup>-2</sup>s<sup>-1</sup> in the highpolated maximum PS II electron transport rate reached<br>2860 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup> in the low-light conditioned state of<br>the leaf and decreased to 1450 µmol m<sup>-2</sup>s<sup>-1</sup> in the high-<br>light conditioned state. The intrinsic quan the leaf and decreased to 1450  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> in the high-<br>light conditioned state. The intrinsic quantum yield  $\varGamma_m$ <br>decreased from 0.41 to 0.23. In these experiments the<br>plateau of PS II light-response curves decre 2860 µmol  $e^{-}$  m<sup>-2</sup>s<sup>-1</sup> in the low-light conditioned state of<br>the leaf and decreased to 1450 µmol m<sup>-2</sup>s<sup>-1</sup> in the high-<br>light conditioned state. The intrinsic quantum yield  $\varUpsilon_m$ <br>decreased from 0.41 to 0.23. In the light conditioned state. The intrinsic quantum yield  $T_{\text{m}}$  decreased from 0.41 to 0.23. In these experiments the plateau of PS II light-response curves decreased almost decreased from 0.41 to 0.23. In these experiments the plateau of PS II light-response curves decreased almost proportionally with the initial slope. Respectively, the initial slope decreased to 0.58 and the maximum rate to plateau of PS II light-response curves decreased almost<br>proportionally with the initial slope. Respectively, the<br>initial slope decreased to 0.58 and the maximum rate to<br>0.51 of the low-light adapted value. These data allow proportionally with the initial slope. Respectively, the<br>initial slope decreased to 0.58 and the maximum rate to<br>0.51 of the low-light adapted value. These data allow us to

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(*a*)



Figure 6. Light-response curves of the initial electron<br>transport into the oxidized plastoquinone pool, calculated<br>from the Q, evolution measurements in figure 5 (open Figure 6. Light-response curves of the initial electron Figure 6. Light-response curves of the initial electron<br>transport into the oxidized plastoquinone pool, calculated<br>from the  $O_2$  evolution measurements in figure 5 (open<br>symbols) and from the fluorescence measurements in transport into the oxidized plastoquinone pool, calculated<br>from the  $O_2$  evolution measurements in figure 5 (open<br>symbols) and from the fluorescence measurements in figure 9<br>(filled symbols). Diamonds, low-light precondi from the  $O_2$  evolution measurements in figure 5 (open<br>symbols) and from the fluorescence measurements in figure<br>(filled symbols). Diamonds, low-light preconditioned leaf.<br>Squares, bigh-light preconditioned leaf. Lines a symbols) and from the fluorescence measurements in figure 9<br>(filled symbols). Diamonds, low-light preconditioned leaf.<br>Squares, high-light preconditioned leaf. Lines are rectangular<br>byparbolae calculated from the mathemati ŏ (filled symbols). Diamonds, low-light preconditioned leaf.<br>Squares, high-light preconditioned leaf. Lines are rectangular hyperbolae calculated from the mathematical model of PS II electron transport with constants given in table 1.<br>state with certainty that both the initial slope and the

state with certainty that both the initial slope and the plateau of the PS II light-response curve decreased in the presence of  $a_1$ , that was mostly of the reversible  $a_2$  type state with certainty that both the initial slope and the plateau of the PS II light-response curve decreased in the presence of  $q_N$  that was mostly of the reversible  $q_I$  type.<br>In the above experiments many pulses were a Exercise of the PS II light-response curve decreased in the essence of  $q_N$  that was mostly of the reversible  $q_I$  type.<br>In the above experiments many pulses were applied vile the leaf was preconditioned to either the low

presence of  $q_N$  that was mostly of the reversible  $q_I$  type.<br>In the above experiments many pulses were applied<br>while the leaf was preconditioned to either the low or<br>high PAD (six different pulse PADs and a series of nin In the above experiments many pulses were applied<br>while the leaf was preconditioned to either the low or<br>high PAD (six different pulse PADs and a series of nine<br>different pulse lengths at each pulse PAD). Correspondwhile the leaf was preconditioned to either the low or<br>high PAD (six different pulse PADs and a series of nine<br>different pulse lengths at each pulse PAD). Correspond-<br>ingly it was not possible to recondition the leaf at high PAD (six different pulse PADs and a series of nine<br>different pulse lengths at each pulse PAD). Correspond-<br>ingly, it was not possible to recondition the leaf at  $2\%$  O<sub>2</sub><br>after each pulse but the leaf was permanentl different pulse lengths at each pulse PAD). Correspondingly, it was not possible to recondition the leaf at 2%  $O_2$  after each pulse but the leaf was permanently at 0.4% ingly, it was not possible to recondition the leaf at 2%  $O_2$ <br>after each pulse but the leaf was permanently at 0.4%<br> $O_2$ , this compromise being the lowest possible  $O_2$  concen-<br>tration at which the *a* and *a* quenchin after each pulse but the leaf was permanently at  $0.4\%$ <br> $O_2$ , this compromise being the lowest possible  $O_2$  concentration at which the  $q_E$  and  $q_I$  quenching were stable and  $O_2$ , this compromise being the lowest possible  $O_2$  concentration at which the  $q_E$  and  $q_I$  quenching were stable and no indications of anaerobiosis were seen. To complete the whole routine, the time of exposure of th tration at which the  $q_E$  and  $q_I$  quenching were stable and<br>no indications of anaerobiosis were seen. To complete the<br>whole routine, the time of exposure of the leaf at 0.4%  $h$ <br>O extended to 90 min. Though reconditionin no indications of anaerobiosis were seen. To complete the<br>whole routine, the time of exposure of the leaf at 0.4%<br>O<sub>2</sub> extended to 90 min. Though reconditioning at the low<br>PAD showed that the PS II inhibition reverted and whole routine, the time of exposure of the leaf at  $0.4\%$ <br>  $O_2$  extended to 90 min. Though reconditioning at the low<br>
PAD showed that the PS II inhibition reverted and the<br>
vields approached  $85-90\%$  of the initial val  $O_2$  extended to 90 min. Though reconditioning at the low<br>PAD showed that the PS II inhibition reverted and the<br>yields approached 85–90% of the initial values, the revers-<br>ible *a*-type quenching evidently dominated in t PAD showed that the PS II inhibition reverted and the yields approached 85–90% of the initial values, the reversible  $q_T$ -type quenching evidently dominated in the total  $q_N$  in these experiments. Thus, these experiments yields approached 85–90% of the initial values, the reversible  $q_T$ -type quenching evidently dominated in the total  $q_N$ <br>in these experiments. Thus, these experiments clearly<br>showed that PS II e<sup>-</sup> transport canacity decr ible  $q_T$ -type quenching evidently dominated in the total  $q_N$ <br>in these experiments. Thus, these experiments clearly<br>showed that PS II e<sup>-</sup> transport capacity decreased in<br>parallel with the reversible ain these experiments. Thus, these experiments clearly showed that PS II  $e^-$  transport capacity decreased in parallel with the reversible  $q_I$ .

# **(g)** *Fluorescence induction and oxygen evolution during multiple turnover pulses: the e¡ect of PS II* nce induction and oxygen<br>le turnover pulses: the e<u>f</u><br>donor side resistance<br>coressones is a reliable que during multiple turnover pulses: the effect of PS II<br>donor side resistance<br>Chlorophyll fluorescence is a reliable quantitative indi-

**c**<br> **c** Chlorophyll fluorescence is a reliable quantitative indicator of steady state  $e^-$  transport rate. Since processes<br>
leading to fluorescence emission are completed within Chlorophyll fluorescence is a reliable quantitative indicator of steady state  $e^-$  transport rate. Since processes leading to fluorescence emission are completed within paperscends there is no reason to doubt that fluores cator of steady state  $e^-$  transport rate. Since processes<br>leading to fluorescence emission are completed within<br>nanoseconds, there is no reason to doubt that fluorescence<br>should not be as good a quantitative indicator of leading to fluorescence emission are completed within nanoseconds, there is no reason to doubt that fluorescence should not be as good a quantitative indicator of  $e^-$  transnanoseconds, there is no reason to doubt that fluorescence<br>should not be as good a quantitative indicator of  $e^-$  trans-<br>port during multiple turnover pulses of ms duration. An<br>important difference between the transient an should not be as good a quantitative indicator of  $e^-$  transport during multiple turnover pulses of ms duration. An important difference between the transient and steady-<br>state process is that  $e^-$  transport in pulses is port during multiple turnover pulses of ms duration. An<br>important difference between the transient and steady-<br>state process is that  $e^-$  transport in pulses is extremely<br>fest compared with the steady state. Under this con important difference between the transient and steady-<br>state process is that  $e^-$  transport in pulses is extremely<br>fast compared with the steady state. Under this condition<br>the accumulation of  $P^+$  may become important. state process is that  $e^-$  tr<br>fast compared with the stee<br>the accumulation of  $P_{680}^+$  t The transport in pulses is extremely<br>the steady state. Under this condition<br> $\frac{1}{680}$  may become important. In the<br>the total O wield from pulses were fast compared with the steady state. Under this condition<br>the accumulation of  $P_{680}^+$  may become important. In the<br>following experiments the total  $O_2$  yield from pulses were following experiments the total  $O_2$  yield from pulses were<br>*Phil. Trans. R. Soc. Lond.* B (2000)



time (ms)<br>Figure 7. Fluorescence-induction curves in (*a*) a low-light and<br>(*b*) a bigh-light adapted sunflower leaf. At the beginning of Figure 7. Fluorescence-induction curves in  $(a)$  a low-light an  $(b)$  a high-light adapted sunflower leaf. At the beginning of the traces fluorescence yield corresponds to  $F'$  and the the traces, fluorescence yield corresponds to  $F'_{o}$  and the a low-light<br>beginning o<br> $\frac{1}{2}$  and the<br> $(-3.06 \text{ V})$ (*b*) a high-light adapted sunflower leaf. At the beginning of<br>the traces, fluorescence yield corresponds to  $F'_{\alpha}$  and the<br>upper-limit of the plot area corresponds to  $F'_{\alpha}$  (= 3.06 V in<br>(*c*) and 1.27 V in (*h*)). T the traces, fluorescence yield corresponds to  $F'_{\text{o}}$  and the<br>upper-limit of the plot area corresponds to  $F'_{\text{m}}$  (= 3.06 V i<br>(*a*) and 1.27 V in (*b*)). The fast increase of fluorescence<br>corresponds to the beginning upper-limit of the plot area corresponds to  $F'_{m}$  (= 3.06 V in (*a*) and 1.27 V in (*b*)). The fast increase of fluorescence corresponds to the beginning of the light pulse. Marked points correspond to fluorescence  $(F)$ (*a*) and 1.27 V in (*b*)). The fast increase of fluorescence<br>corresponds to the beginning of the light pulse. Marked<br>points correspond to fluorescence (*F*<sub>i</sub>) when 3 µmol e<sup>-m<sup>-2</sup><br>have been transferred to reduce bound a</sup> points correspond to fluorescence  $(F_i)$  when 3 µmole<sup>-</sup> m<sup>-2</sup><br>have been transferred to reduce bound acceptors. Further on,<br>free plastoquinone is reduced. Pulse absorbed quantum flux<br>densities (PADs) were (downward for cur have been transferred to reduce bound acceptors. Further on, free plastoquinone is reduced. Pulse absorbed quantum flux densities (PADs) were (downward for curves) 13 500, 10 200, free plastoquinone is reduced. Pulse absorbed quantum flux 6800, 3600, 1720 and 880  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>.

compared with the integral of  $e^-$  transport calculated compared with the integral of  $e^-$  transport calculated<br>from fluorescence induction during the same pulses.<br>Fluorescence was measured with a PAM 101 fluoremeter compared with the integral of  $e^-$  transport calculated<br>from fluorescence induction during the same pulses.<br>Fluorescence was measured with a PAM 101 fluorometer<br> $(H-Wa)z$  Effeltrich Germany) Electron transport rate from fluorescence induction during the same pulses.<br>Fluorescence was measured with a PAM 101 fluorometer (H. Walz, Effeltrich, Germany). Electron transport rate was calculated using the formula (Genty *et al.* 1989). Fluorescence was measured with a PAM 101 fluorometer (H. Walz, Effeltrich, Germany). Electron transport rate was calculated using the formula (Genty *et al.* 1989):

$$
\tilde{\mathcal{J}}_{\mathcal{F}} = a_{\mathcal{II}} Q \frac{F'_{\mathcal{m}} - F(t)}{F'_{\mathcal{m}}},\tag{1}
$$

 $\mathcal{J}_F = a_H Q \frac{m}{F'_m}$ , (1)<br>where  $F'_m$  is light-saturated and  $F(t)$  is time-dependent<br>fluorescence vield during the pulse. The fraction of where  $F'_{m}$  is light-saturated and  $F(t)$  is time-dependent<br>fluorescence yield during the pulse. The fraction of<br>light-absorbed by PS II antenna  $g_{\text{tr}}$  was 0.5 in the low-light where  $F'_{\text{m}}$  is light-saturated and  $F(t)$  is time-dependent<br>fluorescence yield during the pulse. The fraction of<br>light absorbed by PS II antenna,  $a_{\text{II}}$ , was 0.5 in the low-light<br>adapted state but 0.43 gave a bette fluorescence yield during the pulse. The fraction of<br>light absorbed by  $PSII$  antenna,  $a_{II}$ , was 0.5 in the low-light<br>adapted state but 0.43 gave a better fit in the high-light<br>adapted state light absorbed by PS II antenna,  $a_{\text{II}}$ , was 0.5 in the low-light adapted state but 0.43 gave a better fit in the high-light adapted state.

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Figure 8. Total per pulse of electrons calculated from fluorescence related to total per pulse of electrons calculated from  $O_2$  evolution when pulse length was varied from 5 to 160 ms in (a,b) a low light and (c,d) a hi Figure 8. Total per pulse of electrons calculated from fluorescence related to total per pulse of electrons calculated from  $O_2$  evolution when pulse length was varied from 5 to 160 ms in  $(a,b)$  a low light and  $(c,d)$  a hi  $(O_2$  evolution) and figure 7 (chlorophyll fluorescence). Pulse absorbed quantum flux densities (PADs) were 13 500 (diamonds), leaf. Donor side resistance ( $r_d$  in equation (2)) was assumed to be 0 ( $a,c$ ) or 0.00014s m<sup>2</sup> µmol<sup>-1</sup> ( $b,d$ ) (O<sub>2</sub> evolution) and figure 7 (chlorophyll fluorescence). Pulse absorbed quantum flux densities (PAD 10 200 (s

Fluorescence induction transients recorded during long Fluorescence induction transients recorded during long<br>pulses of different intensities in leaves preconditioned to<br>low and bigh PAD are shown in figure 7. In dark-adapted Fluorescence induction transients recorded during long<br>pulses of different intensities in leaves preconditioned to<br>low and high PAD are shown in figure 7. In dark-adapted<br>leaves the induction was a complex curve with a tra pulses of different intensities in leaves preconditioned to<br>low and high PAD are shown in figure 7. In dark-adapted<br>leaves the induction was a complex curve with a transient<br>minimum (data not shown) but adaptation to PAD o Now and high PAD are shown in figure 7. In dark-adapted<br>Cleaves the induction was a complex curve with a transient<br>minimum (data not shown) but adaptation to PAD of leaves the induction was a complex curve with a transient<br>minimum (data not shown) but adaptation to PAD of<br>60  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> or higher eliminated the minimum and<br>the induction became approximately exponential Electro minimum (data not shown) but adaptation to PAD of 60  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> or higher eliminated the minimum and the induction became approximately exponential. Electron transport during the pulses ( $\mathcal{F}_r$  equation (1)) wa 60  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup> or higher eliminated the minimum and<br>the induction became approximately exponential. Electron<br>transport during the pulses ( $\mathcal{J}_F$ , equation (1)) was calcu-<br>lated using simultaneously recorded data the induction became approximately exponential. Electron<br>transport during the pulses  $(\mathcal{J}_F$ , equation (1)) was calcu-<br>lated using simultaneously recorded data points of fluores-<br>cence and pulse PAD and an  $F'$ , value m transport during the pulses  $(\mathcal{F}_F$ , equation (1)) was calculated using simultaneously recorded data points of fluorescence and pulse PAD and an  $F'_{m}$ -value measured at the end of a separate 1s pulse of 13.500 umol qu lated using simultaneously recorded data points of fluorescence and pulse PAD and an  $F'_{\text{m}}$ -value measured at the end of a separate 1s pulse of 13 500 µmol quanta m<sup>-2</sup>s<sup>-1</sup>, corrected for the presence of electron tra  $\mu$  of a separate 1s pulse of 13500  $\mu$ molquantam<sup>-2</sup>s<sup>-1</sup>, cence and pulse PAD and an  $F'_{\text{m}}$ -value measured at the end of a separate 1s pulse of 13500  $\mu$ mol quanta m<sup>-2</sup>s<sup>-1</sup>,<br>corrected for the presence of electron transport rate (ETR)<br>and donor side resistance. This  $F'_{m}$  (3.06 V in figure 7*a* and<br>1.27 V in figure 7*b* corresponds t corrected for the presence of electron transport rate (ETR)<br>and donor side resistance. This  $F'_{\text{m}}$  (3.06 V in figure 7*a* and<br>1.27 V in figure 7*b*, corresponds to the upper limit of the<br>plot area), was bigher than th and donor side resistance. This  $F'_{\text{m}}$  (3.06 V in figure 7*a* and 1.27 V in figure 7*b*, corresponds to the upper limit of the plot area), was higher than that reached at the end of 160 ms pulses in figure 7 because F 1.27 V in figure 7*b*, corresponds to the upper limit of the plot area), was higher than that reached at the end of 160 ms pulses in figure 7, because ETR through PS II was still fast during the pulse plot area), was higher th.<br>160 ms pulses in figure 7, b<br>still fast during the pulse. *Phil. Trans. R. Soc. Lond.* B (2000)

The calculated values of  $\mathcal{J}_F$  were integrated point by The calculated values of  $\mathcal{J}_F$  were integrated point by point to find the total  $e^-$  transport during a pulse of a given length which was then compared with the The calculated values of  $\mathcal{J}_F$  were integrated point by<br>point to find the total  $e^-$  transport during a pulse of a<br>given length, which was then compared with the<br>measured total  $\Omega$ , evolution during the same pulse. P point to find the total  $e^-$  transport during a pulse of a<br>given length, which was then compared with the<br>measured total  $O_2$  evolution during the same pulse. Pulse<br>totals of the calculated  $e^-$  transport and measured  $O$ given length, which was then compared with the measured total  $O_2$  evolution during the same pulse. Pulse totals of the calculated  $e^-$  transport and measured  $O_2$  evolution were proportional to one another when pulse measured total  $O_2$  evolution during the same pulse. Pulse length was increased at constant PAD, but the slope of evolution were proportional to one another when pulse<br>length was increased at constant PAD, but the slope of<br>the relationship (e<sup>-</sup> from fluorescence/e<sup>-</sup> from O<sub>2</sub> evolu-<br>tion  $\mathcal{I}(\mathcal{I})$  was dependent on pulse PAD (f tion,  $\mathcal{J}_{\rm F}/\mathcal{J}_{\rm O}$  was dependent on pulse PAD (figure 8*a,c*).<br>In pulses of low PAD, where ETR was slow,  $\mathcal{J}_{\rm F}/\mathcal{J}_{\rm O}$  was close to one independent of pre-adaptation conditions,<br>but in pulses of high PA was increased at constant PAD, but the slope of<br>tionship (e<sup>-</sup> from fluorescence/e<sup>-</sup> from O<sub>2</sub> evolu-<br> $\sqrt{\mathcal{J}_O}$ ) was dependent on pulse PAD (figure 8*a*,*c*).<br>es of low PAD, where ETR was slow  $\mathcal{J}_C/\mathcal{J}_L$  was the relationship (e<sup>-</sup> from fluorescence/e<sup>-</sup> from O<sub>2</sub> e<br>tion,  $\mathcal{J}_F/\mathcal{J}_O$ ) was dependent on pulse PAD (figure<br>In pulses of low PAD, where ETR was slow,  $\mathcal{J}_F/\mathcal{J}_C$ <br>close to one independent of pre-adaptation co In pulses of low PAD, where ETR was slow,  $\tilde{J}_F/\tilde{J}_O$  was close to one independent of pre-adaptation conditions, but in pulses of high PAD that caused fast e transport close to one independent of pre-adaptation conditions,<br>but in pulses of high PAD that caused fast e transport<br> $\mathcal{J}_F/\mathcal{J}_O$  was much higher than one. Thus, equation (1) well<br>describes the relationship between fluoresce but in pulses of high PAD that caused fast e transport  $\mathcal{J}_F/\mathcal{J}_O$  was much higher than one. Thus, equation (1) well describes the relationship between fluorescence and PS II  $\mathcal{F}_F/\mathcal{F}_O$  was much higher than one. Thus, equation (1) well<br>describes the relationship between fluorescence and PS II<br>e<sup>-</sup> transport during fluorescence induction at physio-<br>logical ETR values, but it progressively describes the relationship between fluorescence and PS II  $e^-$  transport during fluorescence induction at physiological ETR values, but it progressively overestimates  $\tilde{J}_0$  when PAD becomes much higher than physiologi  $e^-$  transport during fluorescence induction at physio-

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ETR increases during short pulses. These results suggest<br>that there is a fraction in  $F' = F^{(t)}$  that is quenched but ETR increases during short pulses. These results suggest<br>that there is a fraction in  $F'_{m} - F(t)$  that is quenched, but<br>not photochemically because it is not accompanied by ETR increases during short pulses. These results suggest<br>that there is a fraction in  $F'_{m} - F(t)$  that is quenched, but<br>not photochemically, because it is not accompanied by<br>corresponding  $e^-$  transport. Quite evidently, t that there is a fraction in  $F'_{m} - F(t)$  that is quenched, but<br>not photochemically, because it is not accompanied by<br>corresponding  $e^{-}$  transport. Quite evidently, this fraction<br>is quenched by  $P^{+}$  the PS II donor pigme not photochemically, because it is not accompanied by corresponding  $e^-$  transport. Quite evidently, this fraction<br>is quenched by  $P_{680}^+$ , the PS II donor pigment that accu-<br>mulates in oxidized form in the presence of corresponding  $e^-$  transport. Quite evidently, this fraction is quenched by  $P_{680}^+$ , the PS II donor pigment that accumulates in oxidized form in the presence of the very fast  $e^-$  transport from OEC to PQ during the h  $e^-$  transport from OEC to PQ during the high-intensity  $e^-$  transport from OEC to PQ during the high-intensity<br>pulses. An empirical formula that describes the relation-<br>ship between fluorescence and  $e^-$  transport in the<br>presence of  $P^+$  can be found from the data presented pulses. An empirical formula that describes the relation-<br>ship between fluorescence and  $e^-$  transport in the<br>presence of  $P_{680}^+$  can be found from the data presented<br>here considering that the amount of  $P^+$  is propor ship between fluorescence and  $e^-$  transport in the presence of  $P_{680}^+$  can be found from the data presented here, considering that the amount of  $P_{680}^+$  is proportional to  $e^-$  transport rate and the proportionalit presence of  $P_{680}^{+}$  can be found from the data presented<br>here, considering that the amount of  $P_{680}^{+}$  is proportional<br>to  $e^{-}$  transport rate and the proportionality constant may<br>be expressed as PS II donor side r here, considering that the amount of  $P_{680}^{+}$  is proportional

$$
\mathcal{J}'_{\mathrm{F}} = \mathcal{J}_{\mathrm{O}} = a_{\mathrm{II}} Q \frac{F'_{\mathrm{m}} - F(t)}{F'_{\mathrm{m}}} \times \frac{1}{1 + a_{\mathrm{II}} r_{\mathrm{d}} Q}.
$$
\n(2)

Compared with equation (1), equation (2) contains and disconsignment  $r$ , which characterizes the donor Compared with equation (1), equation (2) contains an additional parameter  $r_d$ , which characterizes the donor side resistance of PS II per unit leaf area. The quality of Compared with equation (1), equation (2) contains an additional parameter  $r_d$ , which characterizes the donor side resistance of PS II per unit leaf area. The quality of equation (2) for the calculation of feat ETR throug additional parameter  $r_{\rm d}$ , which characterizes the donor side resistance of PS II per unit leaf area. The quality of equation (2) for the calculation of fast ETR through PS II is demonstrated in figure 8*b*,*d*, where one and the equation (2) for the calculation of fast ETR through<br>PS II is demonstrated in figure  $8b,d$ , where one and the<br>same donor side resistance  $r_d$  of 0.00014 µmol<sup>-1</sup>m<sup>2</sup>s was<br>applied for all pulse lengths and PADs and for bot same donor side resistance  $r_d$  capplied for all pulse lengths and high-light adapted states.

# **(h)** *Time- course of PS II electron transport calculated from £uorescence*

(h) Time-course of PS II electron transport<br>calculated from fluorescence<br>Time-courses of electron transport were calculated<br>on fluorescence induction curves applying equation (2) **calculated from fluorescence**<br>Time-courses of electron transport were calculated<br>from fluorescence induction curves applying equation (2) considering the donor side re-reduction time. In figure 9 from fluorescence induction curves applying equation (2) considering the donor side re-reduction time. In figure 9 the data are plotted against the cumulative amount of  $e^-$ <br>transported into the PO pool, an integral of  $\$ considering the donor side re-reduction time. In figure 9<br>the data are plotted against the cumulative amount of  $e^-$ <br>transported into the PQ pool, an integral of  $\mathcal{J}_F$  calculated<br>from all recorded data points. After t the data are plotted against the cumulative amount of  $e^-$ <br>transported into the PQ pool, an integral of  $\mathcal{J}_F$  calculated<br>from all recorded data points. After the rising edge of the<br>pulse, passed, fluorescence, increas transported into the PQ pool, an integral of  $\mathcal{J}_F$  calculated<br>from all recorded data points. After the rising edge of the<br>pulse passed, fluorescence increased immediately and<br>continued to increase (figure 7) Correspon from all recorded data points. After the rising edge of the<br>pulse passed, fluorescence increased immediately and<br>continued to increase (figure 7). Correspondingly, the<br>calculated  $e^-$  transport rate decreased from the beg continued to increase (figure 7). Correspondingly, the calculated  $e^-$  transport rate decreased from the begincontinued to increase (figure 7). Correspondingly, the calculated  $e^-$  transport rate decreased from the beginning of the pulse. This was unexpected since it did not agree with the assumption that  $PO$  reduction had a smal calculated  $e^-$  transport rate decreased from the beginning of the pulse. This was unexpected since it did not agree with the assumption that PQ reduction had a small reverse effect on the  $\Omega$ . reduction due to the more ning of the pulse. This was unexpected since it did not<br>agree with the assumption that PQ reduction had a small<br>reverse effect on the  $Q_A$  reduction due to the more nega-<br>tive redox potential of the latter, but, rather, i agree with the assumption that PQ reduction had a small<br>reverse effect on the  $Q_A$  reduction due to the more nega-<br>tive redox potential of the latter, but, rather, indicated<br>that in the light-adapted state the medium poin tive redox potential of the latter, but, rather, indicated potentials of  $Q_A$  and free PQ are almost equal. The that in the light-adapted state the medium point redox<br>potentials of  $Q_A$  and free PQ are almost equal. The<br>initial  $e^-$  transport rate from OEC to a completely<br>oxidized PO pool  $\mathcal{F}_n$  was obtained as the electron tran potentials of  $Q_A$  and free PQ are almost equal. The<br>initial  $e^-$  transport rate from OEC to a completely<br>oxidized PQ pool,  $J_F$ <sub>i</sub> was obtained as the electron trans-<br>port rate after the first 3 umole<sup>-</sup> m<sup>-2</sup> were trans initial  $e^-$  transport rate from OEC to a completely<br>oxidized PQ pool,  $\mathcal{J}_{F_1}$  was obtained as the electron trans-<br>port rate after the first 3 µmol  $e^-$  m<sup>-2</sup> were transported<br>(shown with a dotted line in figure 9). oxidized PQ pool,  $\mathcal{J}_{\text{Fi}}$  was obtained as the electron transport rate after the first 3 µmol e<sup>-</sup> m<sup>-2</sup> were transported (shown with a dotted line in figure 9). The first 2port rate after the first  $3 \mu$ mol e<sup>-</sup> m<sup>-2</sup> were transported<br>
(shown with a dotted line in figure 9). The first 2-<br>  $3 \mu$ mol e<sup>-</sup> m<sup>-2</sup> were transported at a higher rate, as seen<br>
clearly in figure 9*h* evidently becaus (shown with a dotted line in figure 9). The first 2-<br>3  $\mu$ mole<sup>-</sup> m<sup>-2</sup> were transported at a higher rate, as seen<br>clearly in figure 9*b*, evidently because they reduced  $Q_A$ <br>and  $Q$ , and were not exchanged with the free  $3 \mu$ mol e<sup>-</sup> m<sup>-2</sup> were transported at a higher rate, as seen<br>clearly in figure 9*b*, evidently because they reduced  $Q_A$ <br>and  $Q_B$  and were not exchanged with the free PQ pool.<br>The initial rate of PS II electron transpor clearly in figure 9*b*, evidently because they reduced  $Q_A$ <br>and  $Q_B$  and were not exchanged with the free PQ pool.<br>The initial rate of PS II electron transport  $\tilde{J}_{Fi}$  reached<br>about 2000 umol m<sup>-2</sup>s<sup>-1</sup> for the maximum and  $Q_B$  and were not exchanged with the free PQ pool.<br>The initial rate of PS II electron transport  $\mathcal{J}_{F_i}$  reached<br>about 2000 µmol m<sup>-2</sup>s<sup>-1</sup> for the maximum pulse PAD in<br>the low-light adapted state but was clearly l The initial rate of PS II electron transport  $\mathcal{J}_{\text{Fi}}$  reached<br>about 2000 µmol m<sup>-2</sup> s<sup>-1</sup> for the maximum pulse PAD in<br>the low-light adapted state, but was clearly lower<br>(1000 µmol e<sup>-</sup> m<sup>-2</sup> s<sup>-1</sup>) in the high-light about 2000 µmol m<sup>-2</sup> s<sup>-1</sup> for the maximum pulse PAD i<br>the low-light adapted state, but was clearly lowe<br>(1000 µmol e<sup>-</sup> m<sup>-2</sup> s<sup>-1</sup>) in the high-light adapted state.<br>PS II light-response curves were obtained by plottin e low-light adapted state, but was clearly lower<br>  $(100 \mu \text{mol e}^{-} \text{m}^{-2} \text{s}^{-1})$  in the high-light adapted state.<br>
PS II light-response curves were obtained by plotting<br>  $\mu$  values from figure 9 against pulse PAD (figur

(1000 µmol e<sup>-</sup> m<sup>-2</sup>s<sup>-1</sup>) in the high-light adapted state.<br> **PS II** light-response curves were obtained by plotting  $\mathcal{J}_{\text{Fi}}$  values from figure 9 against pulse PAD (figure 6, closed symbols). The light-response cur PS II light-response curves were obtained by plotting  $\mathcal{J}_{Fi}$  values from figure 9 against pulse PAD (figure 6, closed symbols). The light-response curves of PS II elec- $\mathcal{J}_{\text{Fi}}$  values from figure 9 against pulse PAD (figure 6, closed symbols). The light-response curves of PS II electron transport were rectangular hyperbola, and fit well with the O evolution data. Scattering of  $\mathcal$ closed symbols). The light-response curves of PS II electron transport were rectangular hyperbola, and fit well<br>with the  $O_2$  evolution data. Scattering of  $\tilde{J}_{Fi}$  calculated<br>from fluorescence, however, was considerab tron transport were rectangular hyperbola, and fit well<br>with the  $O_2$  evolution data. Scattering of  $\mathcal{J}_{Fi}$  calculated<br>from fluorescence, however, was considerably less than<br>the scattering of  $\mathcal{I}_c$ , calculated fro with the  $O_2$  evolution data. Scattering of  $\mathcal{J}_{Fi}$  calculated from fluorescence, however, was considerably less than the scattering of  $\mathcal{J}_{oi}$  calculated from  $O_2$  evolution. Since there were 2 umol PS II m<sup>-2</sup> ( from fluorescence, however, was considerably less than<br>the scattering of  $\tilde{\jmath}_{\text{Oi}}$  calculated from  $\text{O}_2$  evolution. Since<br>there were 2 µmol PS II m<sup>-2</sup> (see figure 2), and using the there were  $2 \mu \text{mol} \text{PS II m}^{-2}$  (see figure 2), and using the<br>*Phil. Trans. R. Soc. Lond.* B (2000)



Figure 9. Electron transport rate during light pulses<br>calculated from equation  $(2)$  as a function of cumulative<br>electron transport in a sunflower leaf preconditioned at calculated from equation  $(2)$  as a function of cumulative electron transport in a sunflower leaf preconditioned at calculated from equation (2) as a function of cumulative<br>electron transport in a sunflower leaf preconditioned at<br>(*a*) 60 µmol quanta m<sup>-2</sup> s<sup>-1</sup> and (*b*) 2000 µmol quanta m<sup>-2</sup> s<sup>-1</sup>.<br>Pulse a bsorbed quantum flux densi electron transport in a sunflower leaf preconditioned at<br>
(a) 60 µmol quanta m<sup>-2</sup>s<sup>-1</sup> and (b) 2000 µmol quanta m<sup>-2</sup>s<sup>-1</sup>.<br>
Pulse absorbed quantum flux densities (PADs) were<br>
(downward for curves) 13.500, 10.200, 6800, (a) 60 µmol quanta m<sup>-2</sup>s<sup>-1</sup> and (b) 2000 µmol quanta m<sup>-2</sup>s<sup>-1</sup>.<br>Pulse absorbed quantum flux densities (PADs) were<br>(downward for curves) 13 500, 10 200, 6800, 3600, 1720 and<br>880 µmol m<sup>-2</sup>s<sup>-1</sup>. The initial electron tra 880 µmol m<sup>-2</sup> s<sup>-1</sup>. The initial electron transport rate ( $\tilde{\gamma}_{\text{Fi}}$ ) (downward for curves) 13 500, 10 200, 6800, 3600, 1720 and was read after  $3 \mu$ mol e<sup>-</sup> m<sup>-2</sup> were transported (at the dotted line).

line).<br>extrapolated  $f_{Fm}$ -values, the average electron transfer<br>time from OEC through PSII to PO were calculated to extrapolated  $\mathcal{J}_{F_m}$ -values, the average electron transfer<br>time from OEC through PSII to PQ were calculated to<br>increase from 700 to 1380 us when  $a_r$  increased from the extrapolated  $\mathcal{J}_{Fm}$ -values, the average electron transfer<br>time from OEC through PSII to PQ were calculated to<br>increase from 700 to 1380 us when  $q_N$  increased from the<br>minimum to the maximum time from OEC through PSII to PQ were calculated to increase from 700 to 1380  $\mu$ s when  $q_N$  increased from the minimum to the maximum.

### **(i)** *Modelling PS II electron transport*

Was this increase related to changes on the PS II donor or acceptor side ? Since donor and acceptor side resis-Was this increase related to changes on the PS II donor<br>or acceptor side? Since donor and acceptor side resis-<br>tances are in series, without applying a mathematical<br>model it is difficult to differentiate where and to what or acceptor side? Since donor and acceptor side resistances are in series, without applying a mathematical model it is difficult to differentiate where and to what extent the rate constants for  $e^-$  transfer changed. A tances are in series, without applying a mathematical<br>model it is difficult to differentiate where and to what<br>extent the rate constants for  $e^-$  transfer changed. A<br>mathematical model of PS II electron transport and model it is difficult to differentiate where and to what extent the rate constants for  $e^-$  transfer changed. A mathematical model of PS II electron transport and extent the rate constants for  $e^-$  transfer changed. A<br>mathematical model of PS II electron transport and<br>fluorescence (Laisk *et al.* 1997) considers mutual conver-<br>sions of the four states of PS II  $A - D^{-}A^{-}$   $B - D^{-}A^{+}$ mathematical model of PS II electron tra<br>fluorescence (Laisk *et al.* 1997) considers mu<br>sions of the four states of PS II,  $A = D^{-}A^{-}$ ,<br> $C - D^{+}A^{-}$ ,  $D - D^{+}A^{+}$ , where D is dono  $A^-$ ,  $B = D^-A^+$ , fluorescence (Laisk *et al.* 1997) considers mutual conversions of the four states of PS II,  $A = D^{-}A^{-}$ ,  $B = D^{-}A^{+}$ ,  $C = D^{+}A^{-}$ ,  $D = D^{+}A^{+}$ , where D is donor and A is acceptor. The experimental data were used for the sions of the four states of PS II,  $A = D^{-}A^{-}$ ,  $B = D^{-}A^{+}$ ,  $C = D^{+}A^{-}$ ,  $D = D^{+}A^{+}$ , where D is donor and A is acceptor. The experimental data were used for the identi-<br>fication of the parameters of the model. For an ex  $C = D^+ A^-$ ,  $D = D^+ A^+$ , where D is donor and A is<br>acceptor. The experimental data were used for the identi-<br>fication of the parameters of the model. For an extreme<br>case of infinitely high PAD and completely oxidized PO acceptor. The experimental data were used for the identi-<br>fication of the parameters of the model. For an extreme<br>case of infinitely high PAD and completely oxidized PO

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<sup>a</sup> Absorbed quantum flux density.

Absorbed quantum flux density.<br>the system of budget equations given in Laisk *et al.* (1997)<br>reduces to the following equation describing the the system of budget equations given in Laisk *et al.* (1997) reduces to the following equation, describing the maximum rate of  $e^-$  transport through a PS II complex: reduces to the following equation, describing the<br>  $\uparrow$  maximum rate of e<sup>-</sup> transport through a PS II complex:

$$
\frac{\tau_{\rm m}}{\tau_{\rm d} + \tau_{\rm a}} = 0.75 + 0.25 \left( \frac{\tau_{\rm d} - \tau_{\rm a}}{\tau_{\rm d} + \tau_{\rm a}} \right)^2, \tag{3}
$$

where  $\tau$  is an exponential time constant, d is donor, a is where  $\tau$  is an exponential time constant, d is donor, a is<br>acceptor and m is maximum throughput, the latter deter-<br>mined from experiments. In terms of time constants, a where  $\tau$  is an exponential time constant, d is donor, a is<br>acceptor and m is maximum throughput, the latter deter-<br>mined from experiments. In terms of time constants, a<br>light-response curve expresses as acceptor and m is maximum throu<br>mined from experiments. In term<br>light-response curve expresses as

$$
\tau_j = \tau_q + \tau_m, \tag{4}
$$

where *j* is the PS II e<sup>-</sup> transport rate and *q* is the PS II where *j* is the PS II e<sup>-</sup> transport rate and *q* is the PS II excitation rate. Equation (4) represents a rectangular hyperbola in rates (it is equivalent to the double-reciprocal where *j* is the PS II  $e^-$  transport rate and *q* is the PS II excitation rate. Equation (4) represents a rectangular hyperbola in rates (it is equivalent to the double-reciprocal plot used in enzume kinetics). It states hyperbola in rates (it is equivalent to the double-reciprocal plot used in enzyme kinetics). It states that  $e^-$  transport hyperbola in rates (it is equivalent to the double-reciprocal<br>plot used in enzyme kinetics). It states that  $e^-$  transport<br>rate is proportional to PAD at low PADs but hyperbolically<br>saturates at a rate of  $1/\tau$ , at bigh P plot used in enzyme kinetics). It states that  $e^-$  transport<br>rate is proportional to PAD at low PADs but hyperbolically<br>saturates at a rate of  $1/\tau_m$  at high PADs. The hyperbolic<br>shape of PS II light-response curves is se rate is proportional to PAD at low PADs but hyperbolically<br>saturates at a rate of  $1/\tau_m$  at high PADs. The hyperbolic<br>shape of PS II light-response curves is seen from figure 6,<br>where experimental data are approximated by saturates at a rate of  $1/\tau_m$  at high PADs. The hyperbolic shape of PS II light-response curves is seen from figure 6, where experimental data are approximated by rectangular shape of PS II light-response curves is seen from figure 6,<br>where experimental data are approximated by rectangular<br>hyperbolae. The maximum rate (plateau) of the light-<br>response curves is symmetrically dependent on the dop where experimental data are approximated by rectangular<br>hyperbolae. The maximum rate (plateau) of the light-<br>response curves is symmetrically dependent on the donor<br>and acceptor time constants (equation (3)) and without hyperbolae. The maximum rate (plateau) of the light-<br>response curves is symmetrically dependent on the donor<br>and acceptor time constants (equation (3)) and without<br>additional information it would be impossible to resolve response curves is symmetrically dependent on the donor<br>and acceptor time constants (equation (3)) and without<br>additional information it would be impossible to resolve<br>both time constants separately. This additional inform and acceptor time constants (equation  $(3)$ ) and without additional information it would be impossible to resolve both time constants separately. This additional information was available from fluorescence measurements wh additional information it would be impossible to resolve<br>both time constants separately. This additional informa-<br>tion was available from fluorescence measurements, where<br>excitation quenching by oxidized donor could be fou both time constants separately. This additional informa-<br>
excitation was available from fluorescence measurements, where allowed detection of PS II donor side resistance separately.<br>  $\blacksquare$  excitation quenching by oxidize tion was available from fluorescence measurements, where<br>excitation quenching by oxidized donor could be found<br>and the donor side time constant was calculated indepen-<br>dently from the acceptor side time constant (see forme excitation quenching by oxidized donor could be found<br>and the donor side time constant was calculated indepen-<br>dently from the acceptor side time constant (see figures 7<br>and 8). However, interpretation of those fluorescenc and the donor side time constant was calculated independently from the acceptor side time constant (see figures 7 and 8). However, interpretation of those fluorescence data was critically dependent on the level of fluoresc O dently from the acceptor side time constant (see figures 7<br>and 8). However, interpretation of those fluorescence data<br>was critically dependent on the level of fluorescence and 8). However, interpretation of those fluorescence data<br>was critically dependent on the level of fluorescence<br>emitted from PS II with  $P_{680}^+$ . Two possible cases were<br>considered: when PS II with  $P^+$  either did not

was critically dependent on the level of fluorescence<br>emitted from PS II with  $P_{680}^{+}$ . Two possible cases were<br>considered: when PS II with  $P_{680}^{+}$  either did not emit fluor-<br>escence or emitted it at a level close emitted from PS II with  $P_{680}^+$ . Two possible cases were<br>considered: when PS II with  $P_{680}^+$  either did not emit fluor-<br>escence or emitted it at a level close to  $F_o$ . The donor side<br>resistances obtained from these considered: when PS II with  $P_{680}^+$  either did not emit fluor-<br>escence or emitted it at a level close to  $F_o$ . The donor side<br>resistances obtained from these two cases were considered<br>senarately in the calculations of escence or emitted it at a level close to  $F_o$ . The donor side<br>resistances obtained from these two cases were considered<br>separately in the calculations of the acceptor side resis-<br>tances (time constants) per PS II centre resistances obtained from these two cases were coseparately in the calculations of the acceptor site<br>tances (time constants) per PS II centre (table 1).<br>Data in table 1 show that PS II acceptor site parately in the calculations of the acceptor side resis-<br>nces (time constants) per PS II centre (table 1).<br>Data in table 1 show that PS II acceptor side time<br>nstants increase, approximately, twofold when  $g_{ij}$  is

tances (time constants) per PS II centre (table 1).<br>Data in table 1 show that PS II acceptor side time<br>constants increase approximately twofold when  $q_N$  is<br>induced: from 607 us to 1315 us (2.16 times) when PS II in Data in table 1 show that PS II acceptor side time<br>constants increase approximately twofold when  $q_N$  is<br>induced: from 607 µs to 1315 µs (2.16 times) when PS II in<br> $P^+$  does not emit fluorescence and from 559 µs to 1169  $P_{680}^{+}$  d constants increase approximately twofold when  $q_N$  is induced: from 607 µs to 1315 µs (2.16 times) when PS II in

 $(2.09 \text{ times})$  when it emits fluorescence at a level close to  $\overline{F}$ . Thus, the PS II acceptor side resistance increased  $F_{\rm o}$ . Thus, the PS II acceptor side resistance increased .09 times) when it emits fluorescence at a level close to . Thus, the PS II acceptor side resistance increased (2.09 times) when it emits fluorescence at a level close to  $F_o$ . Thus, the PS II acceptor side resistance increased about twofold in the presence of predominantly reversible  $a_{\sigma}$ -type quenching. Assuming no fluorescen  $F_o$ . Thus, the PS II acceptor side resistance increased<br>about twofold in the presence of predominantly reversible<br> $q_T$ -type quenching. Assuming no fluorescence from  $P_{680}^+$ <br>the donor side time constant increased only about twofold in the presence of predominantly reversible  $q_T$ -type quenching. Assuming no fluorescence from  $P_{680}^+$ <br>the donor side time constant increased only marginally<br>when a obtained its maximum value (from 289 us  $q_T$ type quenching. Assuming no fluorescence from  $P_{680}^+$ <br>the donor side time constant increased only marginally<br>when  $q_N$  obtained its maximum value (from 289 µs to<br> $329 \text{ u}$  or  $114 \text{ times}$ ) but it almost doubled with the donor side time constant increased only marginally<br>when  $q_N$  obtained its maximum value (from 289 µs to<br>329 µs, or 1.14 times), but it almost doubled with the onset when  $q_N$  obtained its maximum value (from 289 µs to 329 µs, or 1.14 times), but it almost doubled with the onset of  $q_N$  when  $P_{680}^+$  was assumed to emit fluorescence at a level close to  $F_0$  (from 361 µs to 615 µs, 329 µs, or 1.14 times), but it almost doubled with the onset<br>of  $q_N$  when  $P_{680}^+$  was assumed to emit fluorescence at a level<br>close to  $F_0$  (from 361 µs to 615 µs, or 1.70 times). Thus, for<br>the interpretation of these of  $q_N$  when  $P_{680}^+$  was assumed to emit fluorescence at a level<br>close to  $F_0$  (from 361 µs to 615 µs, or 1.70 times). Thus, for<br>the interpretation of these fluorescence data the level of<br>fluorescence emitted in  $P^+$ close to  $F_0$  (from 361 µs to 615 µs, or 1.70 times). Thus, for<br>the interpretation of these fluorescence data the level of<br>fluorescence emitted in  $P_{680}^+$  is of crucial importance. If this<br>level was close to  $F_1$ , th the interpretation of these fluorescence data the level of fluorescence emitted in  $P_{680}^+$  is of crucial importance. If this level was close to  $F_o$ , the calculations showed that then the donor side resistance increase fluorescence emitted in  $P_{680}^{+}$  is of crucial importance. If this<br>level was close to  $F_o$ , the calculations showed that then the<br>donor side resistance increased in parallel with the<br>accentor side resistance but if the level was close to  $F_o$ , the calculations showed that then the donor side resistance increased in parallel with the acceptor side resistance, but if the fluorescence was close to donor side resistance increased in parallel with the<br>acceptor side resistance, but if the fluorescence was close to<br>zero, then no changes on the donor side of PS II were<br>detected in parallel with the reversible a. acceptor side resistance, but if the fluoresce<br>zero, then no changes on the donor side<br>detected in parallel with the reversible  $q_1$ . detected in parallel with the reversible  $q_1$ .

### **4. CONCLUSIONS**

Oxygen yield from single turnover flashes and multiple turnover pulses is an informative parameter that charac-Oxygen yield from single turnover flashes and multiple<br>turnover pulses is an informative parameter that charac-<br>terizes the activation-inactivation state and electron<br>transport rates through PS II in intact leaves. The zin turnover pulses is an informative parameter that characterizes the activation-inactivation state and electron<br>transport rates through PS II in intact leaves. The zir-<br>conjum O analyser combined with a flexible gas system terizes the activation-inactivation state and electron<br>transport rates through PS II in intact leaves. The zir-<br>conium  $O_2$  analyser, combined with a flexible gas system,<br>is an appropriate tool for these measurements. Pa transport rates through PS II in intact leaves. The zirconium  $O_2$  analyser, combined with a flexible gas system, is an appropriate tool for these measurements. Parallel conium  $O_2$  analyser, combined with a flexible gas system,<br>is an appropriate tool for these measurements. Parallel<br>recording of chlorophyll fluorescence and  $O_2$  evolution<br>allowed detection of PS II donor side resistanc is an appropriate tool for these measurements. Parallel recording of chlorophyll fluorescence and  $O_2$  evolution allowed detection of PS II donor side resistance separately.<br>Interpreted with the help of a mathematical mo recording of chlorophyll fluorescence and  $O_2$  evolution<br>allowed detection of PS II donor side resistance separately.<br>Interpreted with the help of a mathematical model of<br>PS II electron transport, these data reveal a ful allowed detection of PS II donor side resistance separately.<br>Interpreted with the help of a mathematical model of<br>PS II electron transport, these data reveal a full picture<br>of rate-limiting processes at PS II Interpreted with the help of a m:<br>PS II electron transport, these data<br>of rate-limiting processes at PS II.<br>These experiments showed the PS II electron transport, these data reveal a full picture<br>of rate-limiting processes at PS II.<br>These experiments showed that PS II properties

depend on the type of non-photochemical quenching These experiments showed that PS II properties<br>depend on the type of non-photochemical quenching<br>present. The rapidly induced and rapidly reversible  $q_E$ <br>type (photoprotective) quenching induces changes neither depend on the type of non-photochemical quenching<br>present. The rapidly induced and rapidly reversible  $q_E$ <br>type (photoprotective) quenching induces changes neither<br>in the number of active PS II nor in the PS II maximum present. The rapidly induced and rapidly reversible  $q_E$ <br>type (photoprotective) quenching induces changes neither<br>in the number of active PS II nor in the PS II maximum<br>turnover rate (a very rapidly reversible process was type (photoprotective) quenching induces changes neither<br>in the number of active PS II nor in the PS II maximum<br>turnover rate (a very rapidly reversible process was still detected, but the relaxation time was faster than that of furnover rate (a very rapidly reversible process was still<br>detected, but the relaxation time was faster than that of<br> $F_{\text{m}}$ ). The antenna mechanism of the  $q_{\text{E}}$ -type quenching is<br>therefore confirmed. The more slowl detected, but the relaxation time was faster than that of  $F_m$ ). The antenna mechanism of the  $q_E$ -type quenching is therefore confirmed. The more slowly but still reversible  $q_A$ -type (photoinactivation) quenching induce  $F_m$ ). The antenna mechanism of the  $q_E$ -type quenching is<br>therefore confirmed. The more slowly but still reversible<br> $q_I$ -type (photoinactivation) quenching induced a decrease<br>in the number of active PS II and in the maxi therefore confirmed. The more slowly but still reversible  $q_{\text{I}}$ -type (photoinactivation) quenching induced a decrease<br>in the number of active PS II and in the maximum PS II<br>turnover rate. The latter parameter was an a  $q_{\text{r}}$ -type (photoinactivation) quenching induced a decrease

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the leaf area and its decrease could result from the decrease in the number of  $e^-$  transporting PS II, while the leaf area and its decrease could result from the decrease in the number of  $e^-$  transporting PS II, while the properties of active PS II did not change. This result shows that the type of quenching termed here as decrease in the number of  $e^-$  transporting PS II, while<br>the properties of active PS II did not change. This result<br>shows that the type of quenching termed here as<br>reversible 4, type is different from the state transition the properties of active PS II did not change. This result<br>shows that the type of quenching termed here as<br>reversible  $q_1$  type is different from the state transition<br>related  $q_+$  type and from the sustained  $q_+$  type shows that the type of quenching termed here as reversible  $q_1$  type is different from the state transition related  $q_T$  type, and from the sustained  $q_E$ -type properties. quenching, both of which cannot induce changes in PS II<br>properties.<br>In the introduction it was pointed out that there is a

properties.<br>In the introduction it was pointed out that there is a<br>surprisingly good complementation between photo-<br>chemical and non-photochemical quenching of excitation In the introduction it was pointed out that there is a<br>surprisingly good complementation between photo-<br>chemical and non-photochemical quenching of excitation,<br>which independent of the type of quenching results in an surprisingly good complementation between photo-<br>chemical and non-photochemical quenching of excitation,<br>which, independent of the type of quenching, results in an<br>almost constant excitation lifetime. Such tight interchemical and non-photochemical quenching of excitation,<br>which, independent of the type of quenching, results in an<br>almost constant excitation lifetime. Such tight interwhich, independent of the type of quenching, results in an almost constant excitation lifetime. Such tight inter-<br>relationship between the seemingly different processes<br>encouraged us to look for a single mechanism to expla almost constant excitation lifetime. Such tight inter-<br>relationship between the seemingly different processes<br>encouraged us to look for a single mechanism to explain<br>all three types of PS II quenching one that is based on relationship between the seemingly different processes<br>encouraged us to look for a single mechanism to explain<br>all three types of PS II quenching, one that is based on<br>charge separation. However, the results of this work d encouraged us to look for a single mechanism to explain<br>all three types of PS II quenching, one that is based on<br>charge separation. However, the results of this work did<br>not support the idea of a unique quenching mechanis all three types of PS II quenching, one that is based on<br>charge separation. However, the results of this work did<br>not support the idea of a unique quenching mechanism<br> $\rho_{hysiol.}$  110, 61–71. for  $q_E$  and  $q_I$ , because changes in PS II properties were charge separation. However, the results of this work did detected that paralleled  $q_I$  but no changes in PS II centres accompanied  $q_{\rm E}$ .

This work was supported by research project TBGMR517 and grant 3907 from the Estonian Science Foundation.

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### *Discussion*

**Discussion**<br>C. Critchley (*Department of Botany*, *University of Queensland*,<br>*Australia*) How do you explain the extremely high (e.g. **Discussion**<br>C. Critchley (Department of Botany, University of Queensland,<br>*Australia*). How do you explain the extremely high (e.g.<br>10) O. evolution rates compared to the CO. dependent C. Critchley (*Department of Botany, University of Queensland, Australia*). How do you explain the extremely high (e.g. 10)  $O_2$  evolution rates compared to the  $CO_2$  dependent rates<sup>2</sup>. What is the relative merit of I W *Australia*). How do you explain the extremely high (e.g. 10)  $O_2$  evolution rates compared to the  $CO_2$  dependent rates? What is the relative merit of J. Whitmarsh's proposal of limited access by PS II to the PO pool ag 10)  $O_2$  evolution rates compared to the  $CO_2$  dependent<br>rates? What is the relative merit of J. Whitmarsh's<br>proposal of limited access by PS II to the PQ pool against<br>your argument of requising this possibility i.e. no rates? What is the relative merit of J. Whitmarsh's<br>proposal of limited access by PS II to the PQ pool against<br>your argument of regulating this possibility, i.e. no limit-<br>ations or access to the PQ pool? proposal of limited access by PS II<br>your argument of regulating this p<br>ations or access to the PQ pool?

A. Laisk. The fast  $O_2$  evolution rates were possible in these experiments because electrons were transported A. Laisk. The fast  $O_2$  evolution rates were possible in these experiments because electrons were transported only from  $H.O$  to completely oxidized plastoquipone A. Laisk. The fast  $O_2$  evolution rates were possible in<br>these experiments because electrons were transported<br>only from H<sub>2</sub>O to completely oxidized plastoquinone<br>(PO). The slowly turning around cytochrome *h*, f these experiments because electrons were transported<br>only from  $H_2O$  to completely oxidized plastoquinone<br>(PQ). The slowly turning around cytochrome  $b_6f$ <br>complex was not yet rate limiting. Plastoquinone was (PQ). The slowly turning around cytochrome  $b_6 f$ <br>
complex was not yet rate limiting. Plastoquinone was<br>
oxidized before a pulse was applied and the pulse was so<br>
short that PO became only partially reduced during only from H<sub>2</sub>O to completely oxidized plastoquinone (PQ). The slowly turning around cytochrome  $b_6 f$  complex was not yet rate limiting. Plastoquinone was oxidized before a pulse was applied and the pulse was so complex was not yet rate limiting. Plastoquinone was<br>oxidized before a pulse was applied and the pulse was so<br>short that PQ became only partially reduced during<br>these pulses. The measured O<sub>1</sub> evolution rates characoxidized before a pulse was applied and the pulse was so<br>short that PQ became only partially reduced during<br>these pulses. The measured  $O_2$  evolution rates charac-<br>terize the rate limitations imposed by water splitting a short that PQ became only partially reduced during<br>these pulses. The measured  $O_2$  evolution rates charac-<br>terize the rate limitations imposed by water splitting and<br>PO diffusion these pulses. The measured  $O_2$  evolution rates characterize the rate limitations imposed by water splitting and PQ diffusion. terize the rate limitations imposed by water splitting and

target analysis of picosecond chlorophyll fluorescence kinetics rate of electron transport supported by those PS II that from pea chloroplasts: a new approach to the characterization had access to the PQ pool. The rather g PQ diffusion.<br>Our multiple turnover pulse experiments detected the<br>rate of electron transport supported by those PS II that<br>had access to the PO pool. The rather good fit of the Our multiple turnover pulse experiments detected the<br>rate of electron transport supported by those PS II that<br>had access to the PQ pool. The rather good fit of the<br>PS II light response to a rectangular hyperbola allows PS II light response to a rectangular hyperbola allows had access to the PQ pool. The rather good fit of the PS II light response to a rectangular hyperbola allows<br>one to conclude that the  $e^-$  transport times on the PS II<br>donor and acceptor side were rather similar at all PS PS II light response to a rectangular hyperbola allows<br>one to conclude that the  $e^-$  transport times on the PS II<br>donor and acceptor side were rather similar at all PS II.<br>Thus there was no wide distribution of  $e^-$  trans one to conclude that the  $e^-$  transport times on the PS II<br>donor and acceptor side were rather similar at all PS II.<br>Thus, there was no wide distribution of  $e^-$  transport<br>times at individual PS II. If there was a signifi donor and acceptor side were rather similar at all PS II.<br>Thus, there was no wide distribution of  $e^-$  transport<br>times at individual PS II. If there was a significant<br>portion of PS II with strictly limited access to PO, th Thus, there was no wide distribution of  $e^-$  transport<br>times at individual PS II. If there was a significant<br>portion of PS II with strictly limited access to PQ, these<br>could be detected by fluorescence rise. The fluoresce times at individual PS II. If there was a significant<br>portion of PS II with strictly limited access to PQ, these<br>could be detected by fluorescence rise. The fluorescence<br>induction curves however corresponded well with the portion of PS II with strictly limited access to PQ, these<br>could be detected by fluorescence rise. The fluorescence<br>induction curves, however, corresponded well with the<br>model that considered only a homogenous PS II popula could be detected by fluorescence rise. The fluorescence<br>induction curves, however, corresponded well with the<br>model that considered only a homogenous PS II popula-<br>tion. The presence of a very small fraction of PS II with induction curves, however, corresponded well with the model that considered only a homogenous PS II population. The presence of a very small fraction of PS II with different kinetic characteristics was still possible. model that considered only a homogenous PS II popula-

C. B. Osmond (*Research School of Biological Sciences, Australian National (Research School of Biological Sciences, Australian National University, Australia*). Is the rate of devel-<br>Australian National University, Australia). Is the rate of devel-<br>compent of fast and slow co C. B. Osmond (*Research School of Biological Sciences*, *Australian National University*, *Australia*). Is the rate of development of fast and slow components of  $O_2$  flash yield determined by PS II efficiency? *Australian National University, Austral*<br>opment of fast and slow compor<br>determined by PS II efficiency?

by PS II efficiency?<br>determined by PS II efficiency?<br>A. Laisk. The fast component of non-photochemical<br>quenching  $(a_2)$  is usually termed  $a_2$  (energy-denendent) A. Laisk. The fast component of no<br>quenching  $(q_N)$  is usually termed  $q_E$  (et<br>and it develops within  $30-60s$ . This com quenching  $(q_N)$  is usually termed  $q_E$  (energy-dependent) A. Laisk. The fast component of non-photochemical quenching  $(q_N)$  is usually termed  $q_E$  (energy-dependent) and it develops within 30–60s. This component of  $q_N$  does not induce changes in the number of active PS II as quenching  $(q_N)$  is usually termed  $q_E$  (energy-dependent)<br>and it develops within 30–60s. This component of  $q_N$  does<br>not induce changes in the number of active PS II, as<br>determined from saturating single turnover flashes and it develops within 30–60s. This component of  $q_N$  does<br>not induce changes in the number of active PS II, as<br>determined from saturating single turnover flashes, nor in<br>the maximum PS II turnover rate, as determined fro not induce changes in the number of active PS II, as<br>determined from saturating single turnover flashes, nor in<br>the maximum PS II turnover rate, as determined from<br>saturating multiple turnover pulses. The slower compodetermined from saturating single turnover flashes, nor in<br>the maximum PS II turnover rate, as determined from<br>saturating multiple turnover pulses. The slower compo-<br>nent of a, that was termed reversible inhibitory quenchi the maximum PS II turnover rate, as determined from<br>saturating multiple turnover pulses. The slower compo-<br>nent of  $q_N$  that was termed reversible inhibitory quenching<br> $(q_2)$  develops within 30–60 min of illumination and saturating multiple turnover pulses. The slower component of  $q_N$  that was termed reversible inhibitory quenching  $(q_1)$  develops within 30–60 min of illumination and reverses within about 15–30 min This component of  $q_{\$ nent of  $q_N$  that was termed reversible inhibitory quenching  $(q_1)$  develops within 30–60 min of illumination and reverses within about 15–30 min. This component of  $q_N$  is accompanied by a decreased number of active PS I  $(q_I)$  develops within 30–60 min of illumination and<br>reverses within about 15–30 min. This component of  $q_N$  is<br>accompanied by a decreased number of active PS II and<br>a decreased maximum PS II turnover rate reverses within about 15–30 min. This comp<br>accompanied by a decreased number of acti<br>a decreased maximum PS II turnover rate.

accompanied by a decreased number of active 15 H and<br>a decreased maximum PS II turnover rate.<br>M. Richter (*Universität Mainz, Germany*). Nonphotochemical<br>energy discipation at the PS II antenna and at the PS II a decreased maximum 15 H dumover rate.<br>
M. Richter (*Universität Mainz*, *Germany*). Nonphotochemical<br>
energy dissipation at the PS II antenna and at the PS II<br>
reaction centre including enhanced donor side resistance M. Richter (*Universität Mainz*, *Germany*). Nonphotochemical<br>energy dissipation at the PS II antenna and at the PS II<br>reaction centre including enhanced donor side resistance<br>has been distinguished by the related changes energy dissipation at the PS II antenna and at the PS II<br>reaction centre including enhanced donor side resistance<br>has been distinguished by the related changes in the light reaction centre including enhanced donor side resistance<br>has been distinguished by the related changes in the light<br>intensity dependence of oxygen evolution. This is also<br>possible through the measurement of the  $F$  fluore has been distinguished by the related changes in the light<br>intensity dependence of oxygen evolution. This is also<br>possible through the measurement of the  $F_0$  fluorescence<br>that is exclusively quenched by antenna related intensity dependence of oxygen evolution. This is also<br>possible through the measurement of the  $F_0$  fluorescence<br>that is exclusively quenched by antenna related dissipa-<br>tion but not by processes operating at the reactio possible through the measurement of the  $F_0$  fluorescence<br>that is exclusively quenched by antenna related dissipation but not by processes operating at the reaction centre or by enhanced donor side resistance. Have  $F_0$  measurements been performed and are the data consistent with the results from oxygen measurements? or by enhanced donor side resistance. Have  $F_0$  measure-

the results from oxygen measurements?<br>A. Laisk. *F*<sub>o</sub> measurements were carried out but their<br>interpretation is difficult because of the additional PS I A. Laisk.  $F_0$  measurements were carried out but their<br>interpretation is difficult because of the additional PS I<br>fluorescence component in  $F$ . We are presently working A. Laisk.  $F_0$  measurements were carried out but their interpretation is difficult because of the additional PS I fluorescence component in  $F_0$ . We are presently working on quantification of the PS I fluorescence by me interpretation is difficult because of the additional PS I fluorescence component in  $F_o$ . We are presently working on quantification of the PS I fluorescence by measuring emission spectra at different quenching states fluorescence component in  $F_o$ . We are presentl<br>on quantification of the PS I fluorescence by<br>emission spectra at different quenching states.

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