

While quantum yield and effective quantum yield are reduced in bracts in the full sun environment, the light intensity that triggers photoinhibition or photoprotection (through non-photochemical quenching) in bracts and leaves has not yet been investigated. The following experiments use a controlled light intensity supplied by a PAM 2000 or Imaging PAM fluorometer to measure differences in effective quantum yield, photochemical quenching (qP) and non-photochemical quenching (qN) in leaves and bracts from sun and shade environments. The next experiment (6.5.3) measures the response of leaves and inner bracts from dark adaptation to steady state photosynthesis at a constant low light intensity, while further experiments examine the response of bract tissues to light levels between 0 – 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a PAM 2000 fluorometer (experiment 6.5.4) and between 0 - 461 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with an Imaging PAM fluorometer (experiment 6.5.5) to determine the light intensity above which photoinhibition occurs.

6.5.3 Yield and quenching responses of leaves and bracts at steady state photosynthesis

6.5.3.1 Methods

'Fire and Brimstone' and 'Olympic Flame' waratahs at flower maturity (MF stage) were harvested before dawn, immediately taken to the lab and kept in the dark until measurement. Dark-adapted leaves and bracts were exposed to a photosynthetic (actinic) light intensity of 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A saturating pulse was used to probe the photosynthetic system and determine fluorescence yield, qP and qN every 20 seconds until steady state photosynthesis was attained. Measurements were made on each of two leaves and two bracts for two plants in each treatment.

Data were analysed using the Linear Mixed model option of the REML procedure. The number of saturating pulses, that is, the *Time* of probing PSII function, was introduced

as a fixed effect, as well as a random effect (*Plant/Tissue/Time*) with correlated error structures for *Tissue* (diagonal) and *Time* (AR1). The first order autoregressive structure (AR1) models the random error as linearly dependent on the error associated with previous times.

6.5.3.2 Results

Effective quantum yield responses differed significantly, with interactions between tissue type, time of probing and treatment ($P = 0.011$). Effective quantum yield of shaded inner bracts was significantly higher than that of leaves at most times (Figure 6.33). Significant differences in effective quantum yield of inner bracts in the shade compared to those in the sun were observed at 0 and 20 s (initial illumination) and 80-340 s (towards attainment of steady state photosynthesis).

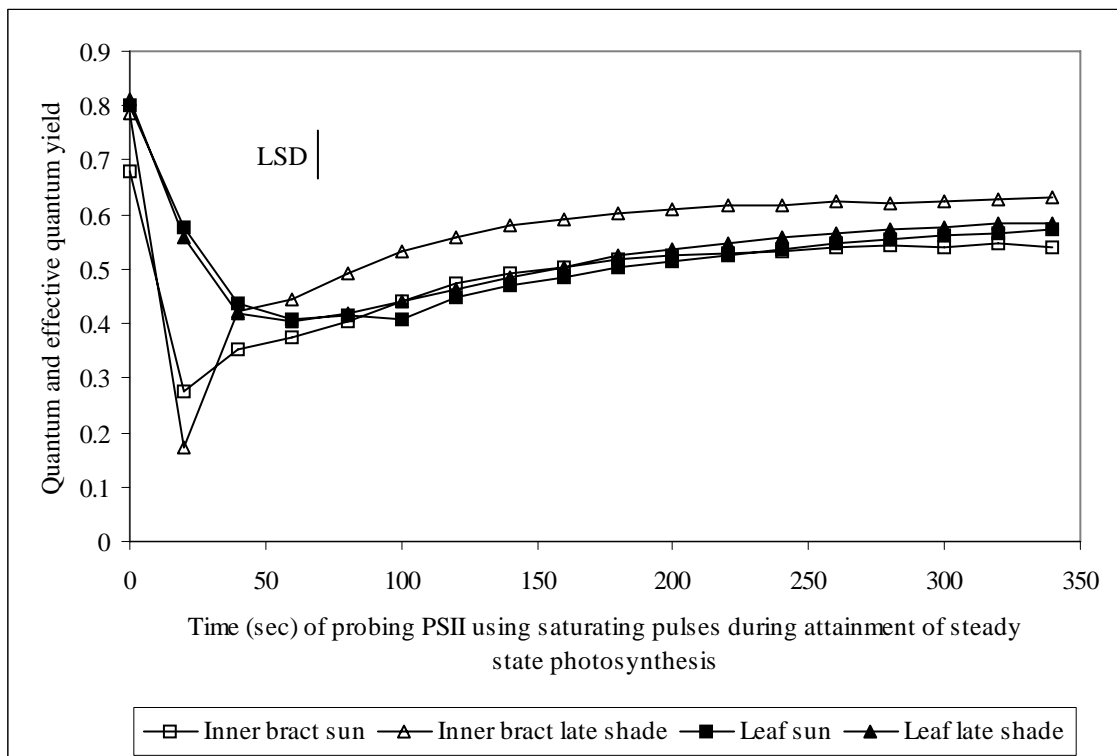


Figure 6.33: Quantum yield (time 0) and effective quantum yield (during light exposure) of inner bracts and leaves at flower maturity grown in full sun or shaded late, pooled across 'Fire and Brimstone' and 'Olympic Flame' cultivars, from dark adaptation (time 0, quantum yield) to steady state photosynthesis (340 s, effective quantum yield) at $80\text{-}100 \mu\text{mol m}^{-2} \text{s}^{-1}$. LSD = 0.068. n = 2 plants of each cultivar in each light treatment.

Analysis of photochemical quenching (qP) showed significant interactions between tissue type, time of probing and cultivar ($P = 0.006$). qP was significantly lower in ‘Olympic Flame’ leaves compared to those of ‘Fire and Brimstone’ (Figure 6.34). However, qP of inner bracts did not vary significantly with cultivar. ‘Olympic Flame’ inner bracts had a significantly higher qP than leaves at steady state photosynthesis (probed at 340 s). ‘Fire and Brimstone’ inner bracts also had a slightly higher qP than leaves, although differences were not significant.

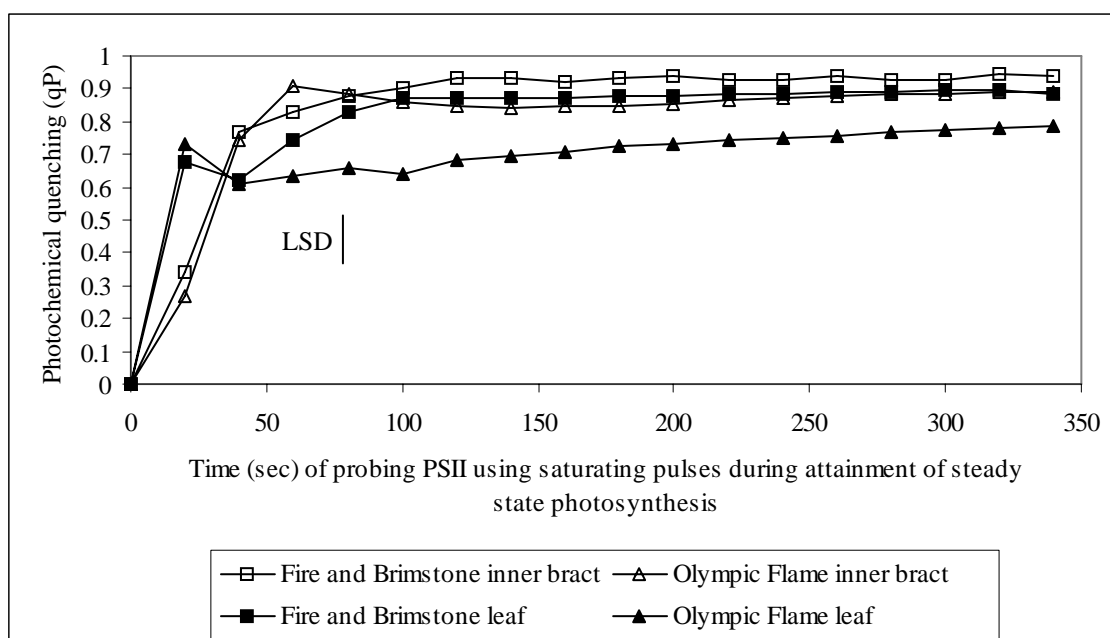


Figure 6.34: Photochemical quenching (qP) in inner bracts and leaves of ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars at flower maturity, pooled across sun and late shade treatments, from dark adaptation (time 0) to steady state photosynthesis (340 s) at $80\text{--}100 \mu\text{mol m}^{-2} \text{s}^{-1}$. LSD = 0.105. $n = 2$ plants of each cultivar in each light treatment.

Non-photochemical quenching (qN) showed significant interactions between tissue type and time, and cultivar and time (both $P < 0.001$). Leaves had a higher capacity for qN than bracts at most times of probing, although differences at steady state photosynthesis were not significant (Figure 6.35). ‘Olympic Flame’ tissues had a significantly lower qN than ‘Fire and Brimstone’ from 80 to 340 s, as steady state photosynthesis was achieved (Figure 6.36).

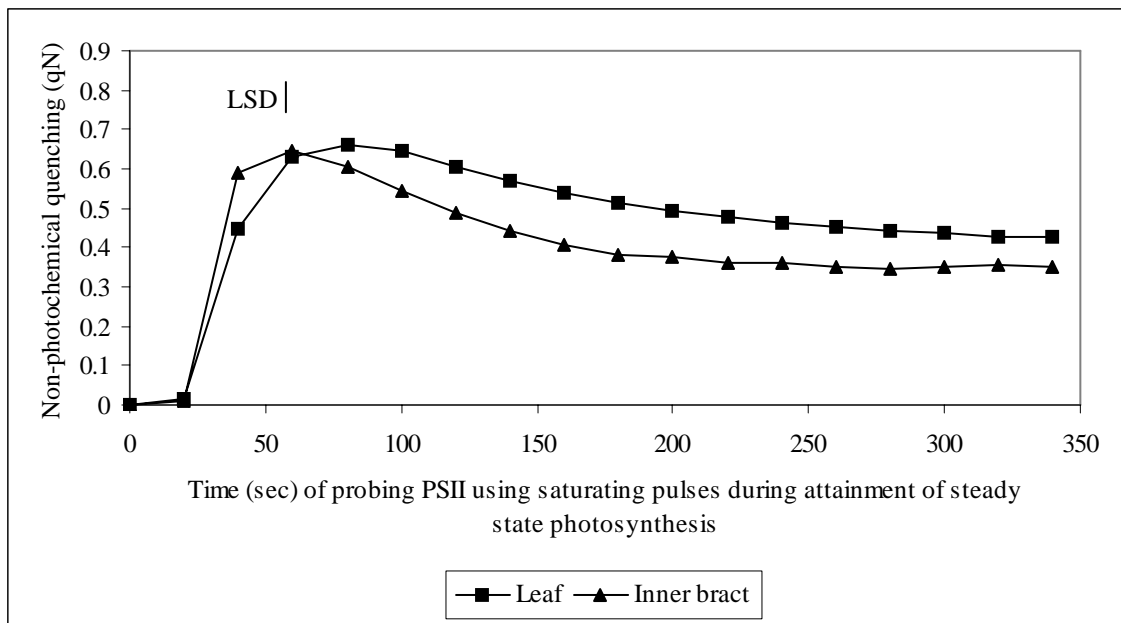


Figure 6.35: Non-photochemical quenching (qN) of leaves and inner bracts, pooled across sun and late shade treatments and cultivars ‘Fire and Brimstone’ and ‘Olympic Flame’ at flower maturity, from dark adaptation (time 0) to steady state photosynthesis (340 s) at 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. LSD = 0.075. n = 2 plants of each cultivar in each light treatment.

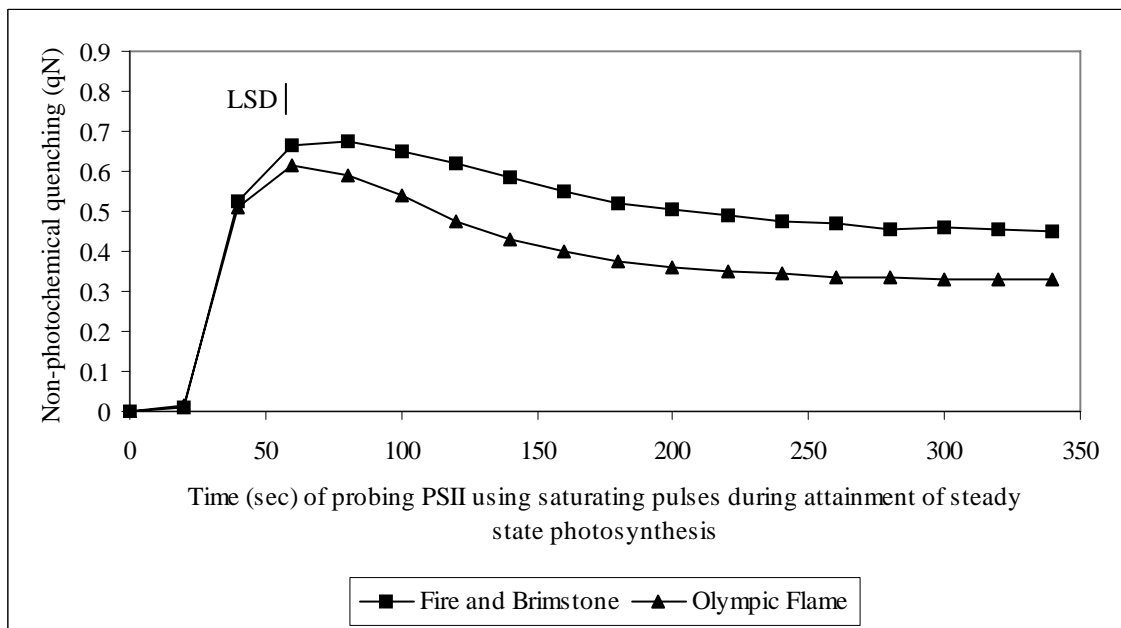


Figure 6.36: Non-photochemical quenching (qN) of ‘Fire and Brimstone’ and ‘Olympic Flame’ cultivars at flower maturity, pooled across leaf and inner bract tissues and sun and late shade treatments, from dark adaptation (time 0) to steady state photosynthesis (340 s) at 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. LSD = 0.075. n = 2 plants of each cultivar in each light treatment.

Differences in leaf and bract responses evident at a low light intensity (experiment 6.5.3) are likely to be more prominent when tissues are exposed to increasing light intensities. The response of leaves and inner bracts grown in different light environments (sun or late shade) to photosynthetically active radiation (PAR) from 0-300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was investigated in the following experiment (6.5.4).

6.5.4 Yield and quenching responses of leaves and bracts (PAM 2000 measurements)

6.5.4.1 Methods

The same tissue was used as described for experiment 6.5.3. Dark-adapted leaves and bracts were subjected to a sequence of light pulses (PAR of 0-300 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Effective quantum yield, qP and qN were determined with each pulse of light on each of two leaves and two bracts for two plants in each treatment.

Data were analysed using the Linear Mixed model option of the REML procedure. The PAR was introduced as a fixed effect, as well as a random effect (*Plant/Tissue/PAR*) with correlated error structures for *Tissue* (diagonal) and *Time* (diagonal).

6.5.4.2 Results

Effective quantum yield responses showed a significant interaction between tissue type and PAR ($P < 0.001$). The effective quantum yield of leaves was significantly higher than that of bracts at light intensities of 0 - 16 and 202 - 303 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.37). The most significant reductions in effective quantum yield were observed between 0 and 13 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in leaves and inner bracts and between 202 and 303 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in inner bracts.

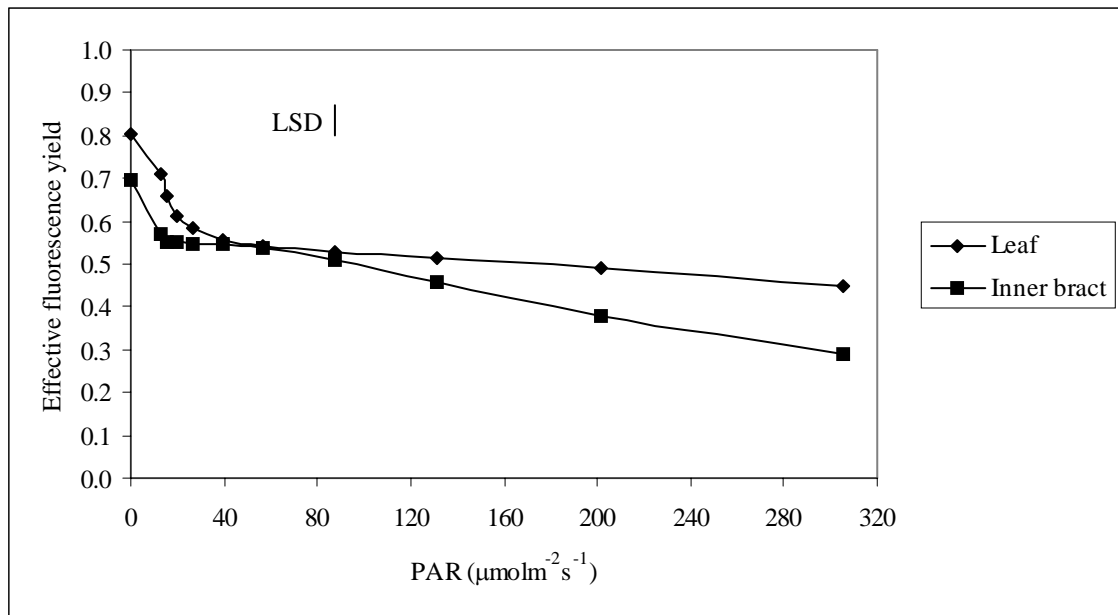


Figure 6.37: Effective fluorescence yield (F_v'/F_m') of leaves and bracts from 0-300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, pooled for 'Fire and Brimstone' and 'Olympic Flame' cultivars at flower maturity and grown in full sun or shaded late in flower development. LSD = 0.068. n = 2 plants of each cultivar in each light treatment.

Photochemical (qP) and non-photochemical (qN) quenching responses showed significant interactions between tissue type and PAR (qP $P = 0.026$, qN $P = 0.018$), and cultivar and PAR (qP $P = 0.003$, qN $P < 0.001$). Photochemical (qP) and non-photochemical (qN) quenching were not significantly different between leaves and bracts, although qN of leaves increased more rapidly than that of bracts (Figure 6.38). The most significant increases in non-photochemical quenching (qN) were noted between 13 and 57 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 132 and 306 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in inner bracts and between 13 and 20 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 202 and 306 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in leaves (Figure 6.38). Significant differences in non-photochemical quenching (qN) of 'Fire and Brimstone' and 'Olympic Flame' cultivars were observed from 132 to 306 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.39). Photochemical quenching (qP) of 'Fire and Brimstone' increased over time, with a significant increase from 57 to 306 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In contrast, qP of 'Olympic Flame' decreased more steadily over time, with a significant decrease from 15 to 306 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

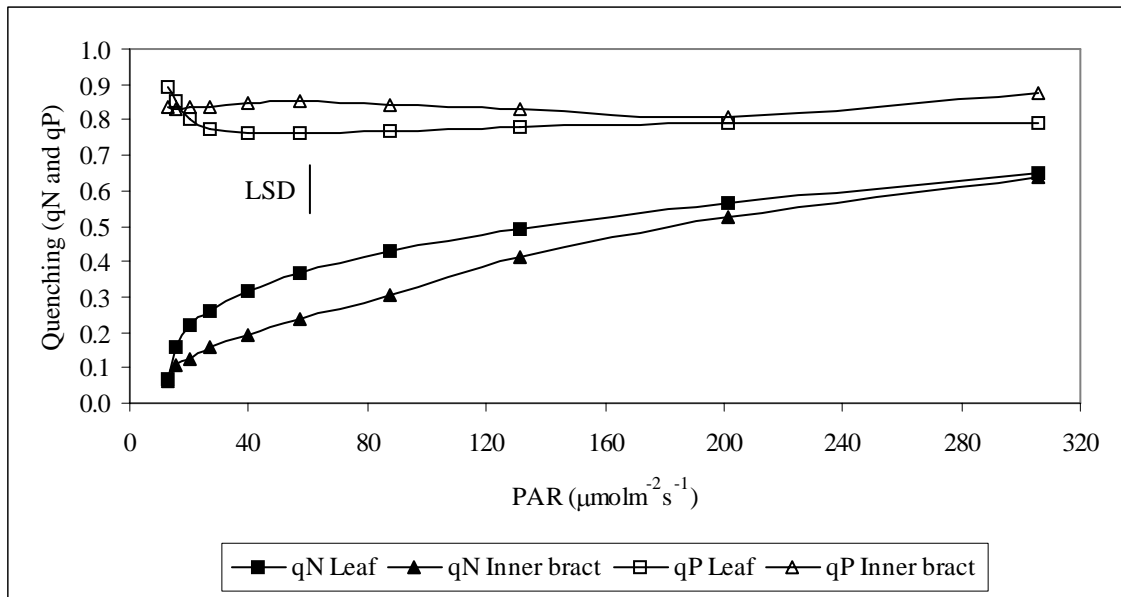


Figure 6.38: Photochemical (qP) and non-photochemical (qN) quenching of inner bracts and leaves at flower maturity, pooled for 'Fire and Brimstone' and 'Olympic Flame' cultivars grown in full sun or shaded late in flower development, over light intensities of 0-306 $\mu\text{molm}^{-2}\text{s}^{-1}$ (PAR, photosynthetically active radiation). LSD = 0.142. n = 2 plants of each cultivar in each light treatment.

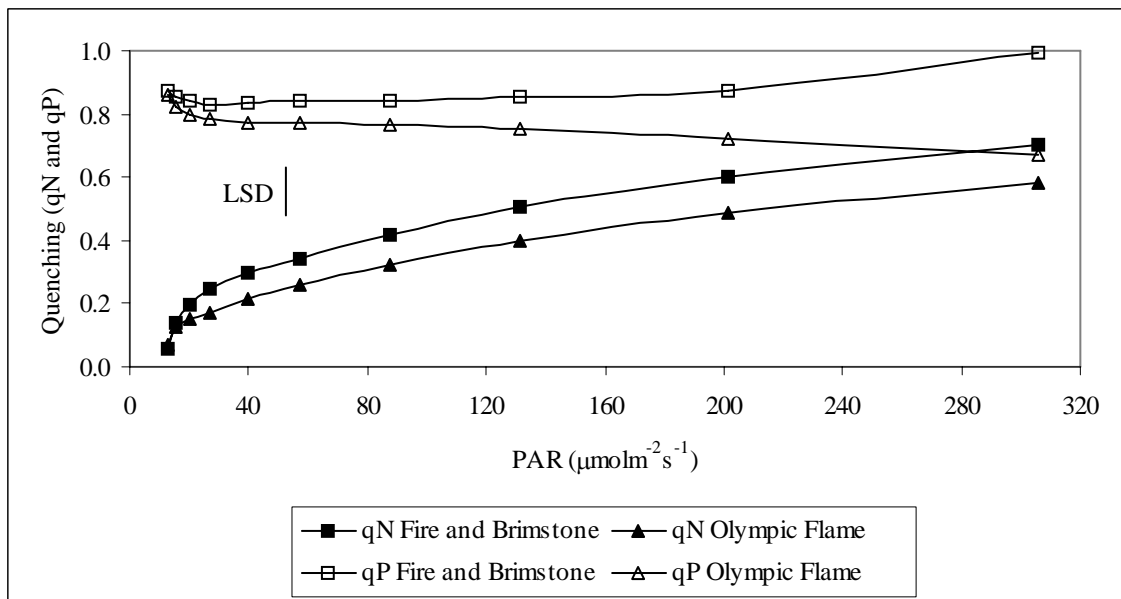


Figure 6.39: Photochemical (qP) and non-photochemical (qN) quenching of 'Fire and Brimstone' and 'Olympic Flame' tissues at flower maturity, pooled across inner bracts and leaves and sun and late shade treatments, and measured over light intensities of 0-306 $\mu\text{molm}^{-2}\text{s}^{-1}$ (PAR, photosynthetically active radiation). LSD = 0.111. n = 2 plants of each cultivar in each light treatment.

Since a significant reduction in effective quantum yield was evident in bracts but not leaves at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, a similar experiment (6.5.5.) was conducted using the Imaging PAM fluorometer to examine variability in the same parameters (effective quantum yield, photochemical and non-photochemical quenching) of leaves and inner bracts at higher light intensities ($0\text{-}461 \mu\text{mol m}^{-2} \text{s}^{-1}$).

6.5.5 Variability in yield and quenching responses of leaves and bracts (Imaging-PAM fluorescence measurements)

6.5.5.1 Method

Waratahs used were the same as those for other Imaging PAM measurements in Experiment 6.4, that is, 'Olympic Flame' waratahs at the JO (juvenile open) stage. Dark-adapted leaves and bracts were subjected to a sequence of light pulses (PAR of $0\text{-}461 \mu\text{mol m}^{-2} \text{s}^{-1}$ for photoinhibition and $461\text{-}61 \mu\text{mol m}^{-2} \text{s}^{-1}$ for recovery). Effective quantum yield, qP and qN were determined with each pulse of light on one typical leaf and bract in each treatment (except for sun bracts, where two samples were measured as variability was expected to be greater), due to availability of Imaging PAM on one day only during the flowering season. Data were collected to give an indication of variability in each tissue type, so statistical analysis was not conducted. Results are presented as values for each of seven to eight areas of interest, defined as in Experiment 6.4.

6.5.5.2 Results

Effective quantum yield, photochemical quenching (qP) and non-photochemical quenching (qN) results are presented as images for one sun leaf, one shade leaf, one sun bract and one shade bract exposed to PAR of 1, 41, 101, 176, 291 and $461 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figures 6.40 - 6.43, respectively). Near infra-red (NIR) images in Figure 6.40 - 6.43

show leaf or bract margins and regions of low chlorophyll (browning). Numerical data for both photoinhibition and recovery corresponding to chosen areas of interest for each tissue and treatment, and measurements for an additional sun bract, are presented in Appendix Figures A4.4-A4.6a-e.

Effective quantum yield (F_v'/F_m') of shade bracts decreased to between 0 and 0.1 by $461 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.43), while effective quantum yield of sun bracts dropped to this level at about $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.42). The effective quantum yield of leaves in the shade or sun dropped to 0.15 at $461 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figures 6.40 and 6.41), and was less variable than that of bracts.

Photochemical quenching (qP) dropped sharply in shade bracts, with values of zero (black areas within bract margin in Figure 6.43) for some samples at $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and all samples by $461 \mu\text{mol m}^{-2} \text{s}^{-1}$. Sun bracts had a qP of zero for some areas throughout the light exposure, with greater variability in the light intensity required for qP to drop to zero. qP of shade and sun leaves dropped to about 0.3 at $461 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Non-photochemical quenching (qN) reached a saturation value in sun bracts of about 0.9 at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (pink areas in Figure 6.42), while shade bracts reached a similar saturation at about $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.43). Shade leaves reached saturation of about 0.8 at $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.41), while sun leaves reached a saturation value of 0.9 at about $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 6.41).

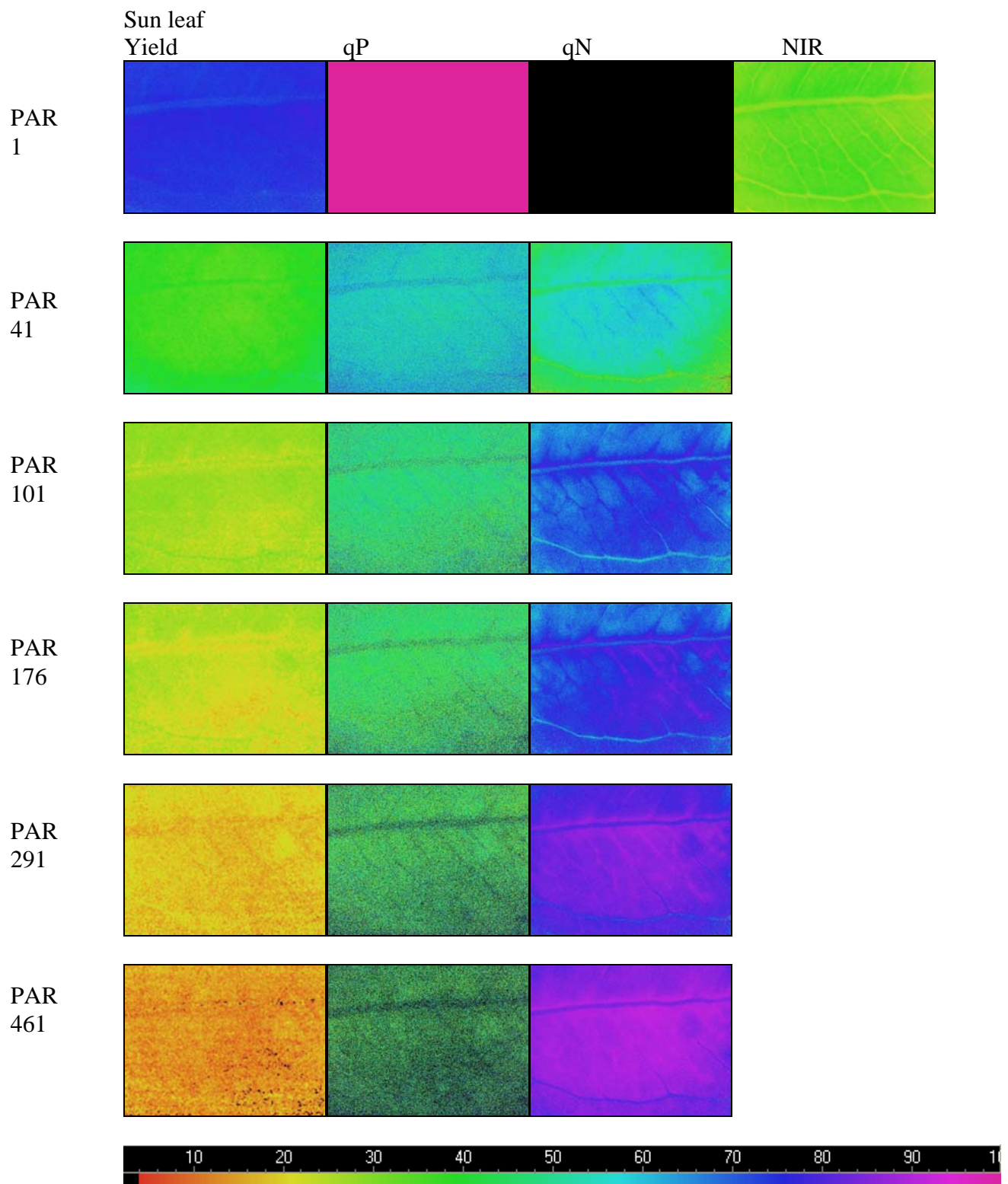


Figure 6.40: Effective quantum yield, photochemical (qP) and non-photochemical quenching (qN) and near infra-red (NIR) chlorophyll fluorescence images of sun-exposed 'Olympic Flame' waratah leaf tissue at the juvenile open (JO) stage of development, exposed to photosynthetically active radiation (PAR) between 1- 461 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

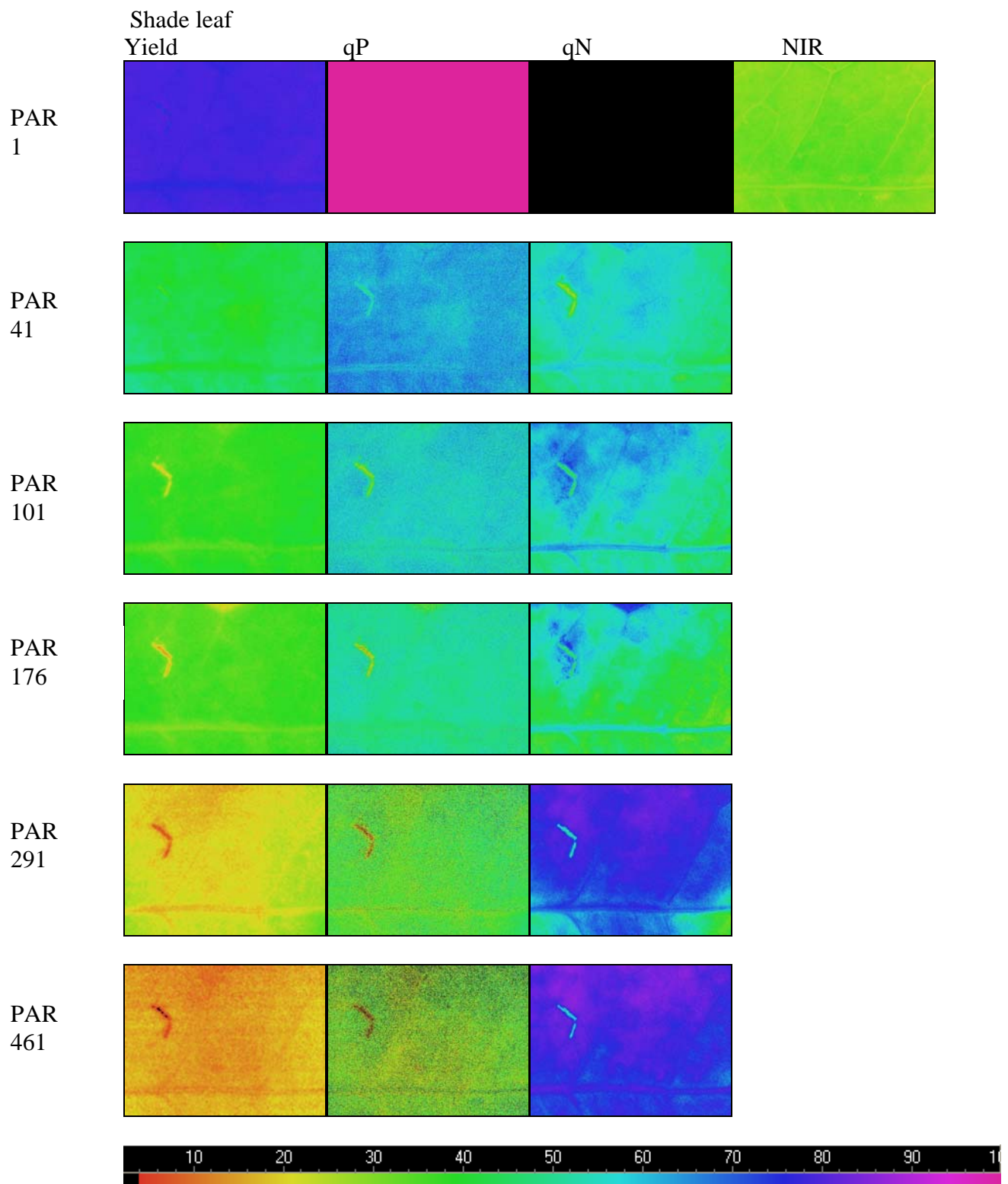


Figure 6.41: Effective quantum yield, photochemical (qP) and non-photochemical quenching (qN) and near infra-red (NIR) chlorophyll fluorescence images of shaded 'Olympic Flame' waratah leaf tissue at the juvenile open (JO) stage of development, exposed to photosynthetically active radiation (PAR) between 1- 461 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

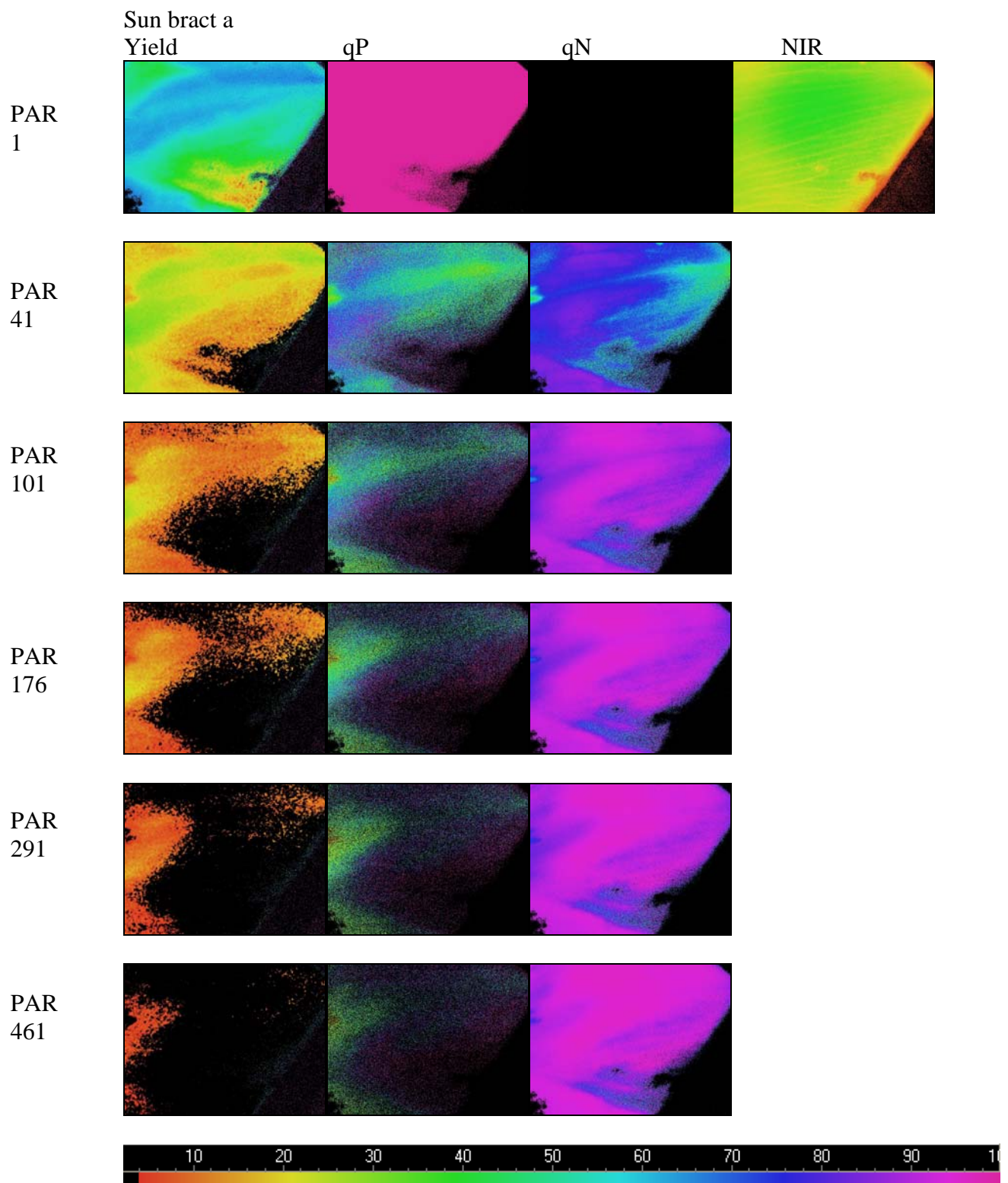


Figure 6.42: Effective quantum yield, photochemical (qP) and non-photochemical quenching (qN) and near infra-red (NIR) chlorophyll fluorescence images of sun-exposed 'Olympic Flame' waratah bract tissue at the juvenile open (JO) stage of development, exposed to photosynthetically active radiation (PAR) between 1- 461 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

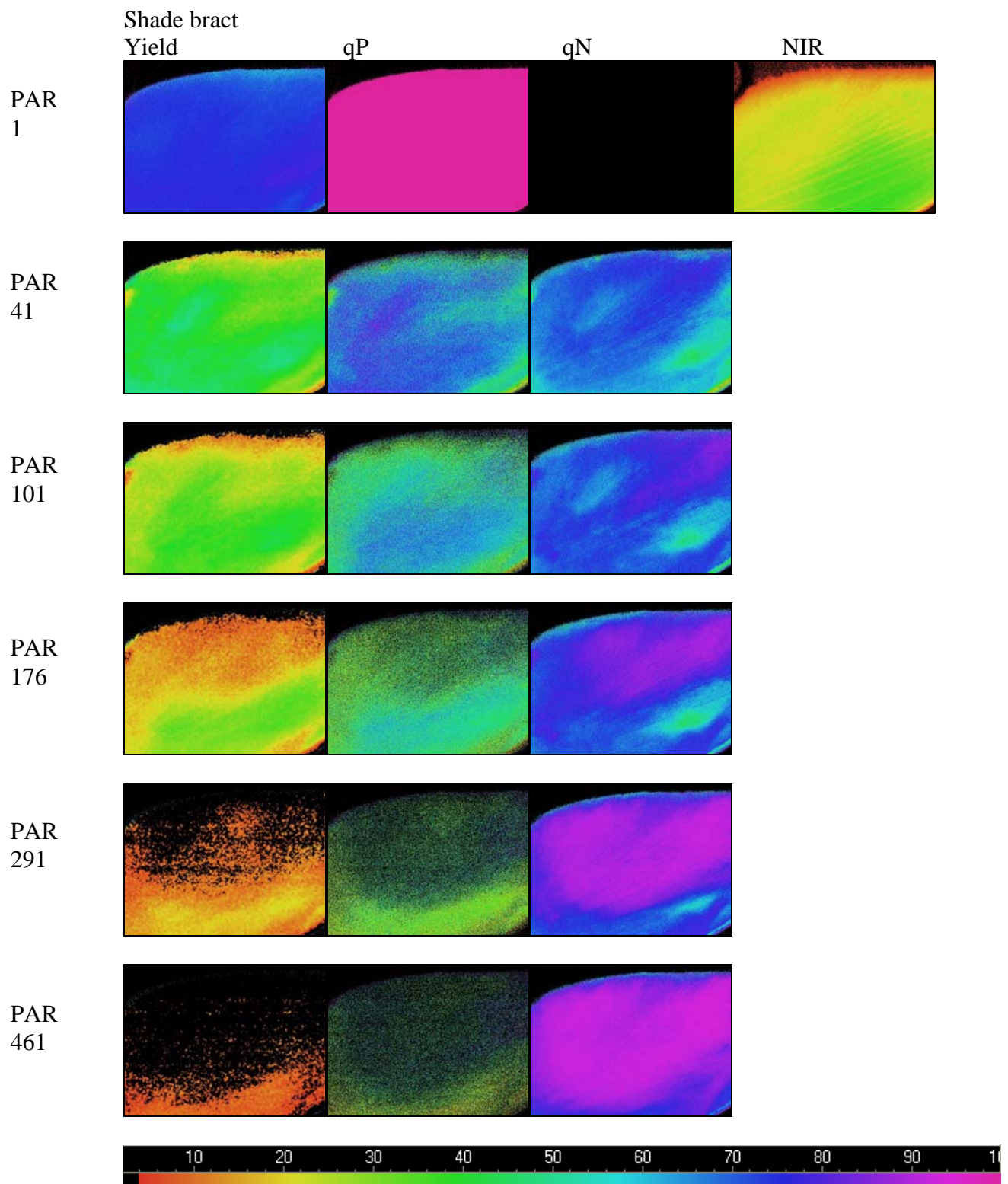


Figure 6.43: Effective quantum yield, photochemical (qP) and non-photochemical quenching (qN) and near infra-red (NIR) chlorophyll fluorescence images of shaded 'Olympic Flame' waratah bract tissue at the juvenile open (JO) stage of development, exposed to photosynthetically active radiation (PAR) between 1- 461 $\mu\text{mol m}^{-2} \text{s}^{-1}$.