April 2018, PSU-Phuket.

Workshop on How to Publish Papers in International Journals

Coordinator : Dr. Raymond J. Ritchie

How to make a poster for a conference.

Growth of Photosynthetic Bacteria (*Rhodopseudomonas palustris*) on Cooking Oils

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Abstract

Large volumes of food waste heavily loaded with cooking oil and liquid waste cooking oil are produced by restaurants, hotels and street food vendors. Much of the oil finds its way to waste treatment plants and drainages where oil and grease are environmentally damaging and costly to deal with. Photoheterotrophic growth of *Rhodopseudomonas palustris* (photosynthetic bacteria) (CGA 009) was used to decompose different types of cooking oil (palm and soybean oils; cooked and uncooked palm oil) under anaerobic and microaerobic conditions. Photosynthesis can be easily measured in *Rhodopseudomonas* using PAM technology (Pulse Amplitude Modulation Fluorometry): Optimum irradiance ≈ 284 μmol photon m^{-2} s^{-1} and maximum photosynthetic electron transport rate 341 μmol e^- g^{-1} BChl *a* s^{-1}. *Rhodopseudomonas* used cooking oils as the sole carbon sources grown anaerobically in PM media with ammonia as the nitrogen source. *Rhodopseudomonas* grew equally well on fresh unused and on used palm oil as well as soybean oil and so used cooking oil is not toxic. Digestion of palm oil and soybean oil was much greater if 0.5% ethanol was added. The small amount of ethanol added apparently formed oil/ethanol micelles in the cell suspension which accelerated digestion of the palm oil and soybean oil. *Rhodopseudomonas* can produce small volumes of hydrogen gas from biodegradation of oil but only under nitrogen fixing conditions (anaerobic: no ammonia) but N-fixing cells grew slowly. A two-stage anaerobic digestion using yeast as the first stage, followed by a *R. palustris* digestion was tested. The yeast-step removed much of the reducing sugar and proteins and partially broke down some of the cooking oil. The second-stage *Rhodopseudomonas* photofermentation grew well taking advantage of the ethanol produced by the yeast but produced very little or no H_2 because of its organic nitrogen content.

Keywords: *Rhodopseudomonas*; Photosynthetic Bacteria; Cooking Oil; Biodegradation; Photosynthesis using PAM Fluoromtery.

Phongiarus, N., Suyaphat C., Naiyana Srichai, N., **Ritchie, R.J.** Growth of photosynthetic bacteria (*Rhodopseudomonas palustris*) on cooking oils. **Asia Pacific Phycological Forum,** Hotel Pullman Kuala Lumpur Bangsar, Kuala Lumpur, 8-13th October 2017. SB13, p 258.

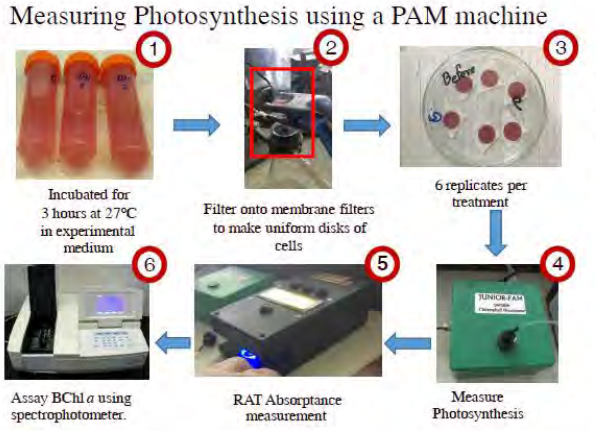

Fig. 1 Measuring photosynthesis of the photosynthetic bacterium using a Pulse Amplitude Modulation (PAM) Fluorometer
1. The cultures grown in PM medium (5ml) were filtered onto 6 membrane filters to produce a uniform disk of cells covering a known surface area. Sets of 6 disks were prepared for each experimental treatment. Disks kept in petri dishes with moist filter paper to prevent dessication.
2. Measure Photosynthetic Electron Transport Rate (ETR) of the photosynthetic bacterium using PAM fluorometry (Ritchie and Runcie, 2013; Ritchie and Mekjinda, 2015).
3. RAT machine in blue light mode is used to measure absorptance (Ritchie and Runcie 2014).
4. Measure Bacteriochlorophyll spectrophotometrically to quote ETR on BChl *a* basis.

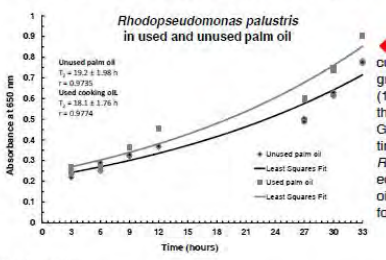

Fig. 2 Comparison of growth curves of *Rhodopseudomonas* cells grown on unused fresh Palm Oil (10 ppt) and used Palm Oil (10 ppt) that had been used to fry chicken. Growth was followed as $A_{650\ nm}$ *vs.* time (h) using a spectrophotometer *Rhodopseudomonas* will grow equally well on unused palm oil and oil that has been used for frying food (t_2 ≈ 18 to 19 hours).

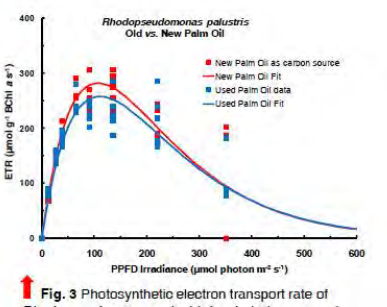

Fig. 3 Photosynthetic electron transport rate of *Rhodopseudomonas palustris* incubated on unused and used palm oil as a carbon source for 3 hours in PM medium. Cells had been grown in modified PM medium with unused Palm Oil as the sole carbon source.

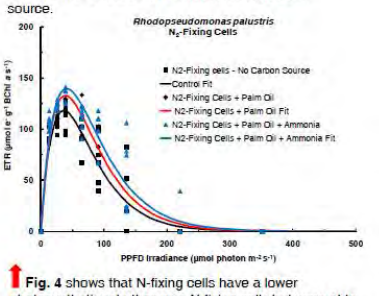

Fig. 4 shows that N-fixing cells have a lower photosynthetic rate than non-N fixing cells but were able to use Palm oil as a carbon source but did not respond quickly to added ammonia.

Highlights
- Liquid waste cooking oil is a major environmental problem,
- *Rhodopseudomonas* metabolises fresh and used Palm oil,
- *Rhodopseudomonas* can also digest Soy Bean Oil,
- Cooking oils can be broken down anaerobically or microaerobically.

References

(1) Ritchie RJ, Runcie JW (2013). Measurement of the Photosynthetic Electron Transport Rate in an Anoxygenic Photosynthetic Bacterium *Afifella* (Rhodopseudomonas) *marina* using PAM Fluorometry. Photochem. Photobiol. 89: 370-383.
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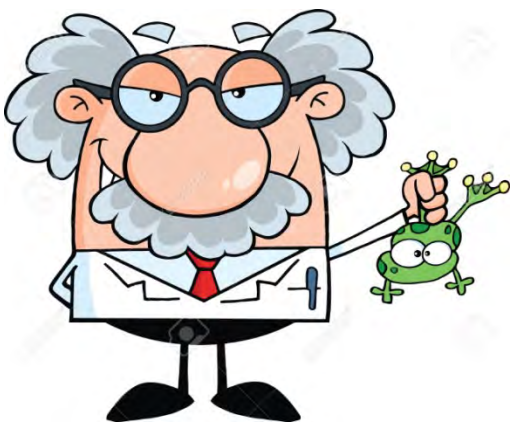
(3) Ritchie RJ, Mekjinda N (2015). Measurement of photosynthesis using PAM technology in a purple sulphur bacterium *Thermochromatium tepidum* (Chromatiaceae). Photochem.Photobiol. 91: 350-358. DOI: 10.1111/php.12413



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Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia aarhiza*.

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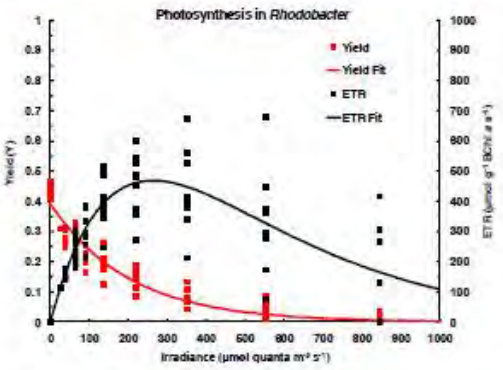
Summary

An arsenic resistant nitrogen fixing photosynthetic bacterium found to live inside *Wolffia aarhiza* plants has been cultured and identified as a *Rhodopseudomonad* species, most likely a strain of *Rhodobacter capsulatus*. It has BChl *a* as its primary photosynthetic pigment and has spectral properties typical of a Rhodopseudomonad. Blue-diode-based PAM (Pulse Amplitude Modulation) technology can be used to measure the photosynthetic electron transport rate (ETR) of the organism. The absorptance of the *Rhodobacter* films on glass fibre discs was measured and used to calculate actual ETR as mol e^- g^{-1} BChl *a* s^{-1}. ETR *vs.* Irradiance (E) curves fitted the waiting-in-line model ($ETR = (ETR_{max} \times E/E_{opt}) \times \exp(1-E/E_{opt})$). Yield (Y) was only ≈ 0.3 to 0.4. *Rhodobacter* saturates at about 250 to 350 µmol photons m^{-2} s^{-1} or ≈ 15% sunlight and shows photoinhibition at high irradiances (overall E_{opt} was 298 ± 7.36 µmol quanta m^{-2} s^{-1}; ETR_{max} = 642 ± 10.6 µmol e^- g^{-1} BChl *a* s^{-1}; Alpha (α) = 6.05 ± 0.200 e^- photon^{-1} m^2 g^{-1} BChl *a*). Photosynthetic performance was much worse in Low-P medium (n = 108, overall E_{opt} 158± 15.4 µmol quanta m^{-2} s^{-1}; ETR_{max} = 194 ± 13.5 µmol e^- g^{-1} BChl *a* s^{-1}; α = 3.30 ± 0.400 e^- photon^{-1} m^2 g^{-1} BChl *a*). *Rhodobacter* is resistant to As (V) toxicity up to at least 1 mol m^{-3} in high and low P medium. The Ki for As(III) in High and Low-P are not significantly different: overall mean was 497 ± 100 mmol m^{-3} but there is a threshold effect below about 200 mmol m^{-3} As(III). Fe(II) and As(III) did not appear to act as electron sources but thiosulphate did act as an electron source for photosynthesis. $Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.

Keywords: Photosynthetic bacteria, *Rhodobacter*, anoxygenic photosynthesis, integrating sphere spectrophotometry, PAM fluorometry.

Fig. 1 *Wolffia* is a genus of very simple flowering plants (Angiosperms). They are the smallest known flowering plants. The very simple anatomy of *Wolffia* helps in the analysis and interpretation of arsenic uptake studies. No roots, stems or leaves or xylem and phloem. The very small size is also an advantage in the laboratory.
Wolffia is somewhat resistant to arsenic but is more resistant to Arsenate (AsV) than to Arsenite (AsIII) (ref. 4). *Wolffia* cells are able to interconvert As(III) and As(V) but cannot convert it into volatile methyl arsines.
Arsenic accumulation in *Wolffia* is important in Thailand because it is used as a vegetable ("Khai-nam").



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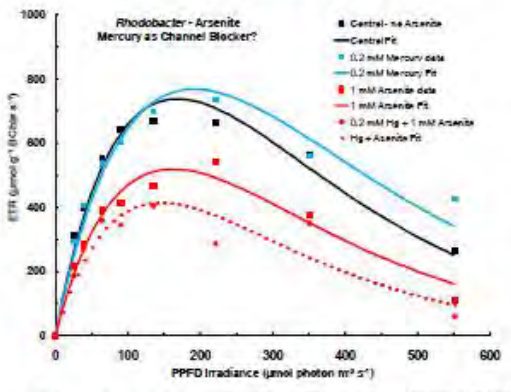

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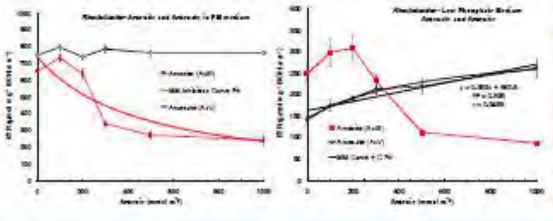

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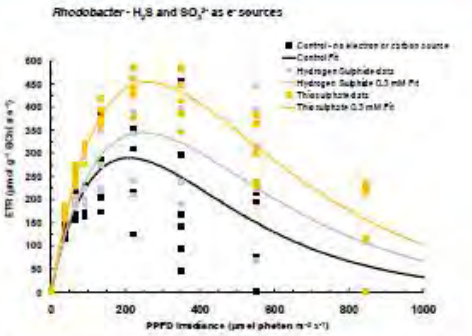

Fig. 5 Photosynthetic electron transport rate of *Rhodobacter* incubated in electron source-free PM medium (control) and supplied with Na_2S or thiosulphate (300 mmol m^{-3}). H_2S marginally increased the photosynthetic ETR and so was being used as an electron source for photosynthesis. Thiosulphate stimulated ETR by more than 50% and so was being used as an electron source. Similar experiments using Fe(II) as a potential e^- source showed that *Rhodobacter* could not use Fe(II) as an electron source unlike most PS Bacteria (Refs 1,2,3)

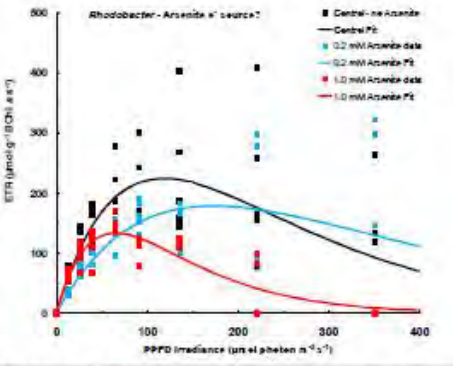

Fig. 6 Photosynthetic electron transport rate of *Rhodobacter* incubated in electron source-free PM medium (control) and supplied with 0.2 mol m^3 As(III) and 1 mol m^3 As(III). As(III) did not increase the photosynthetic ETR and so As(III) could not be used as an electron source for photosynthesis (cf. Ref 5)

Conclusions

- **Arsenite (As(III)) is toxic but Arsenate (As(V)) has no short-term inhibitory effects. Response to As(V) is different under high P compared to low-P conditions.**
- **$Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.**
- **As(III) does not act as an electron source for photosynthesis in *Rhodobacter*.**

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(1) Ritchie RJ, Runcie JW (2013). Measurement of the Photosynthetic Electron Transport Rate in an Anoxygenic Photosynthetic Bacterium *Azolla* (Rhodopseudomonas) *marina* using PAM Fluorometry. Photochem. Photobiol. 89: 370-383.
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Ritchie R.J. and Nakphet, S. (2017) Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia arrhiza*. **Aquafluo II** at University of Technology-Sydney, Sydney, NSW, Australia, 4 to 8th December 2017.

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Underline who is presenting the poster.

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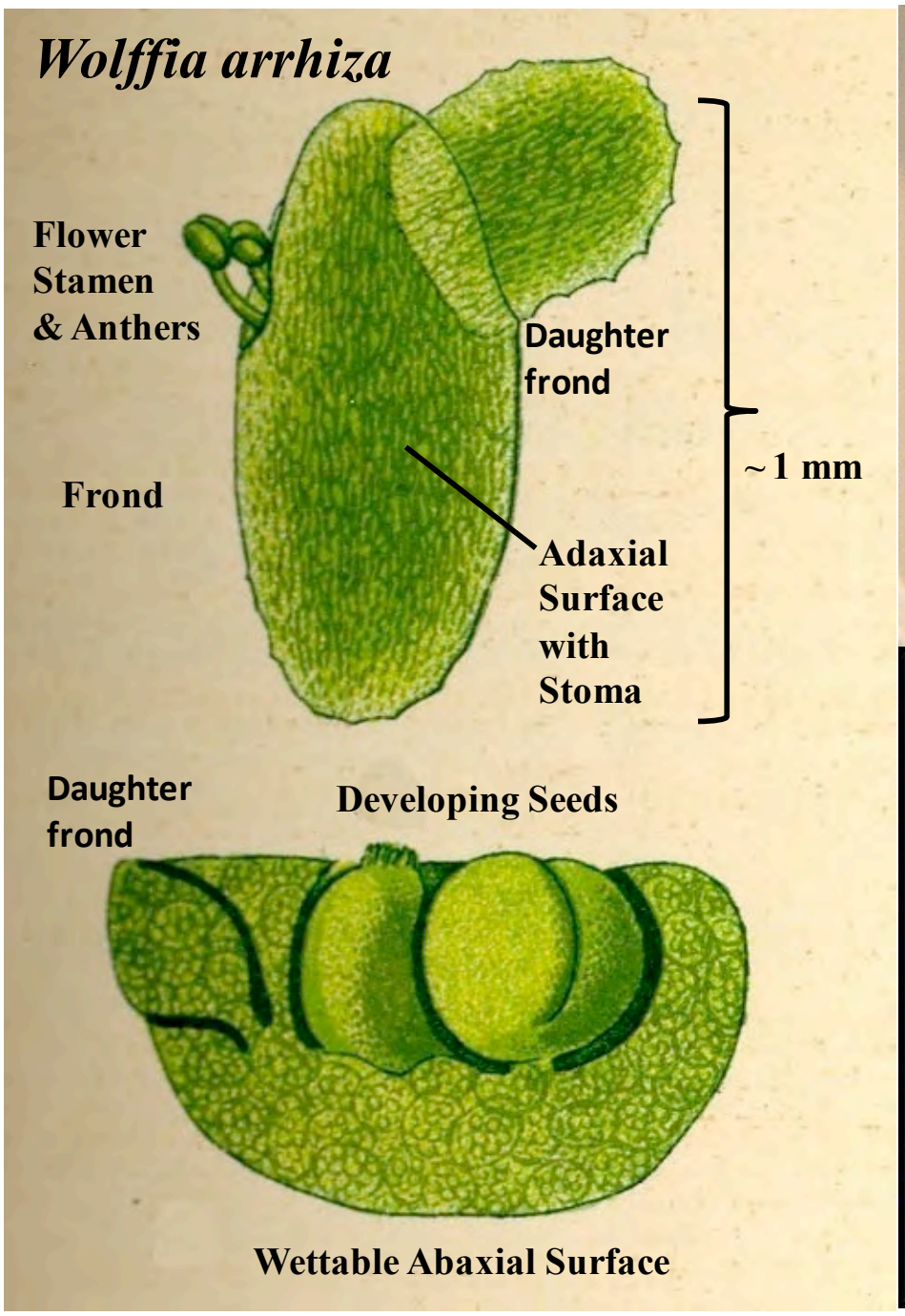
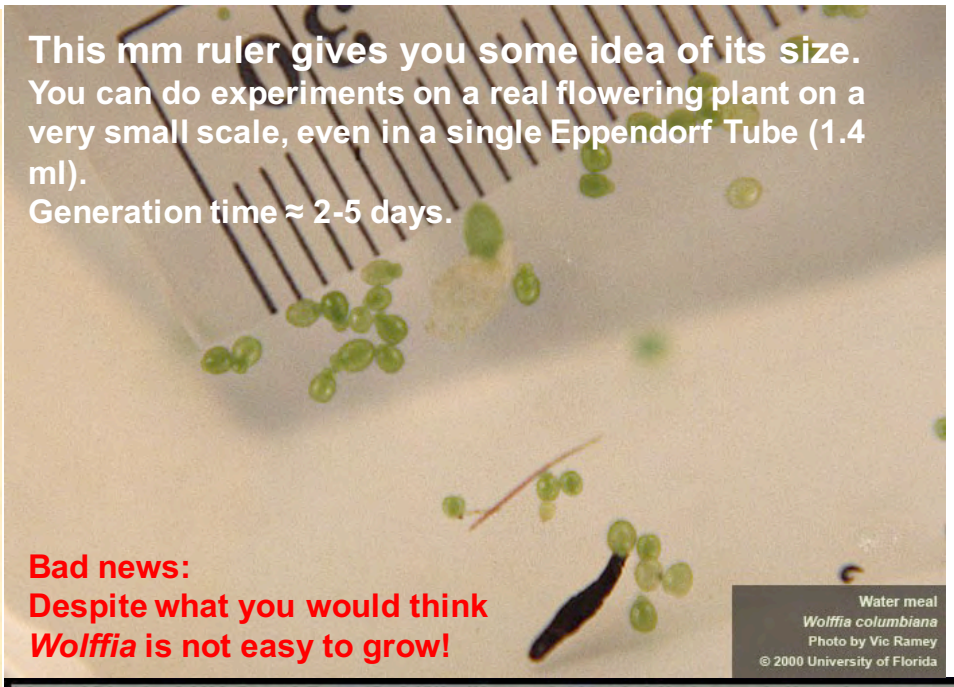
Pretty Pictures





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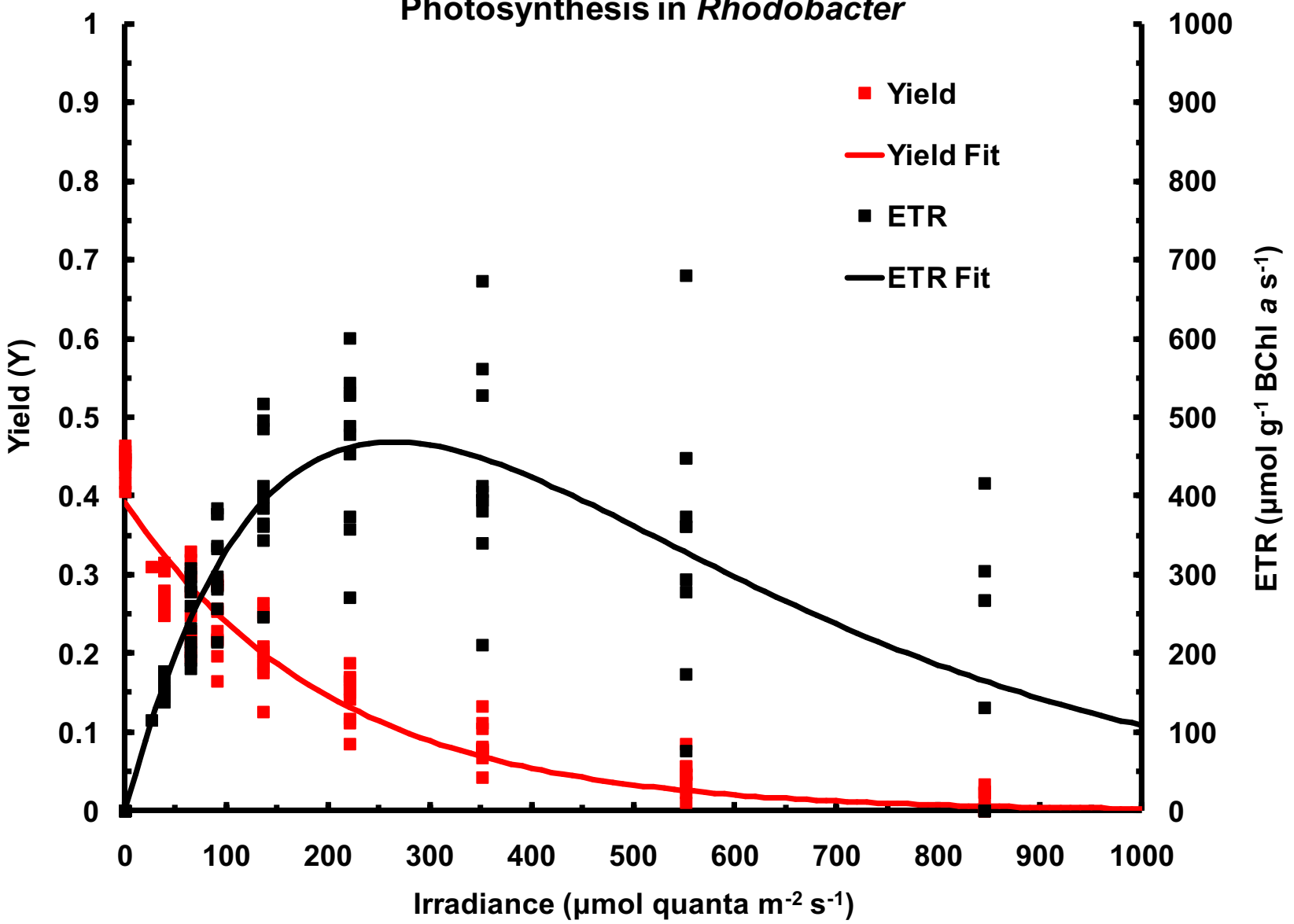

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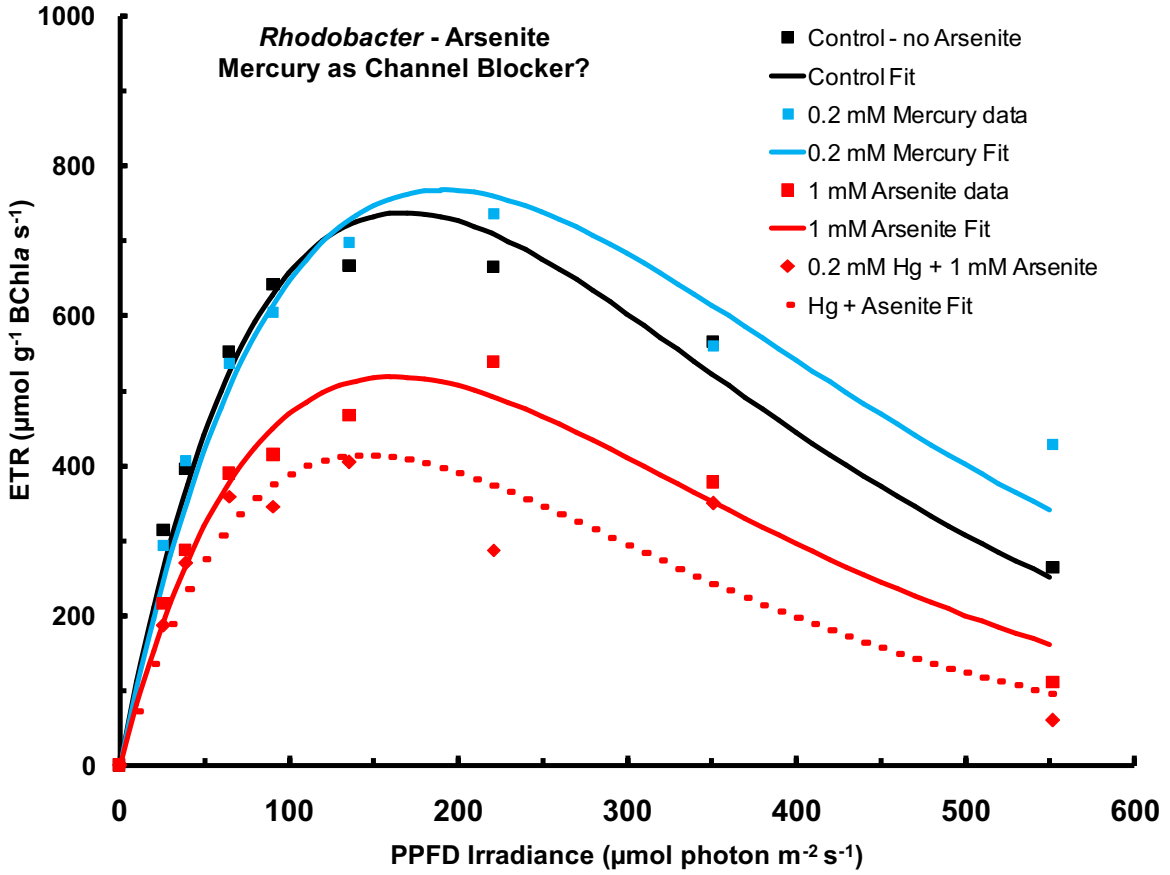

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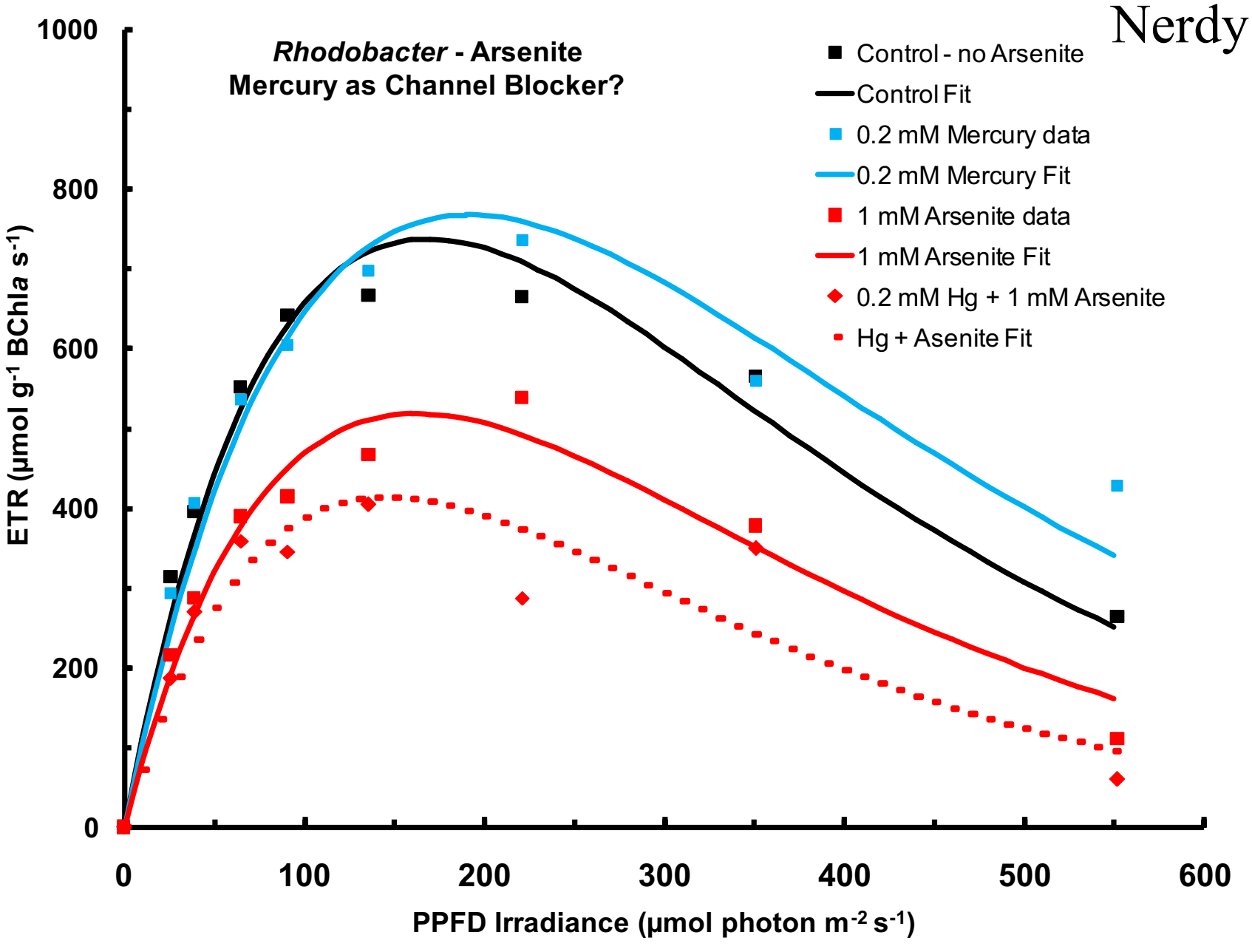

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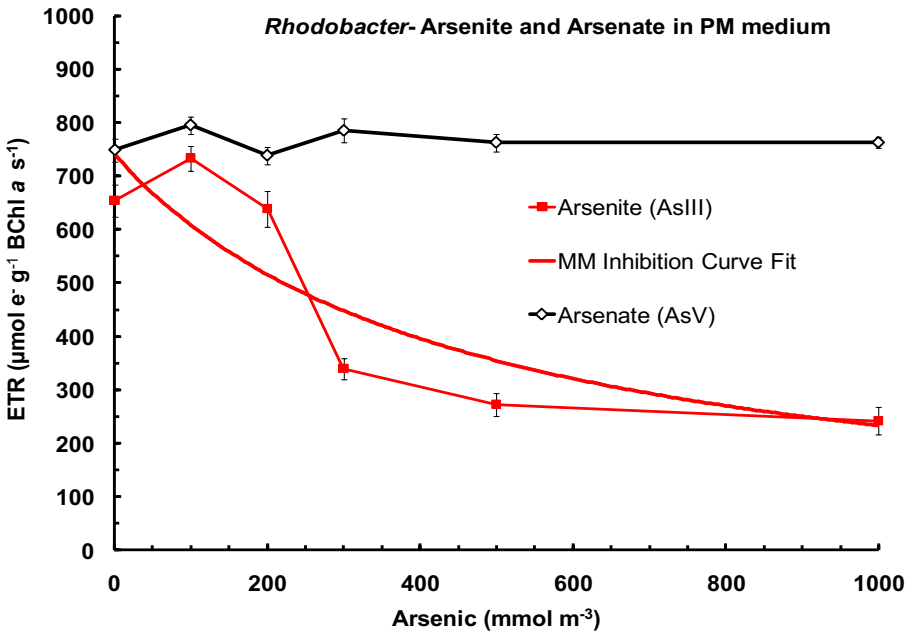

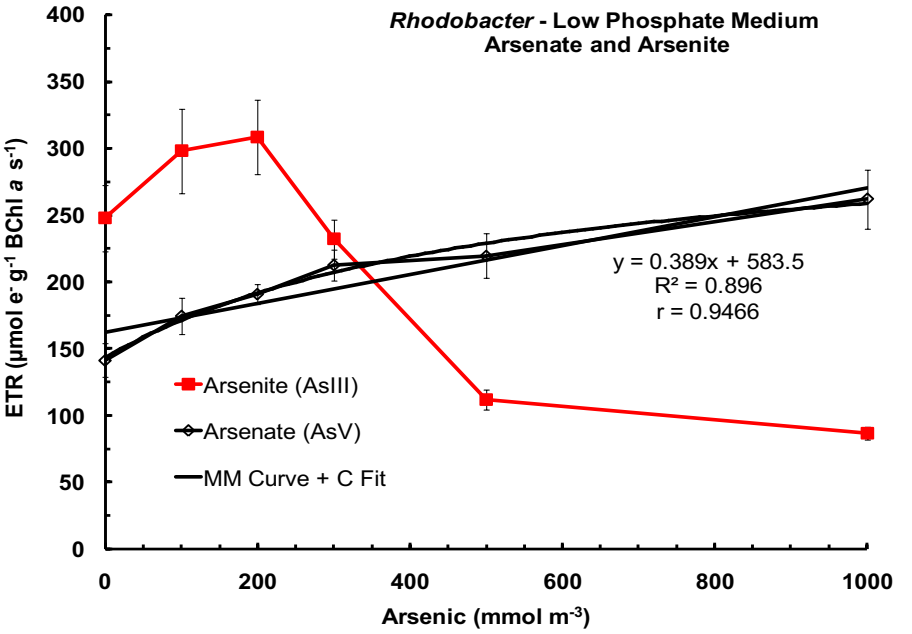

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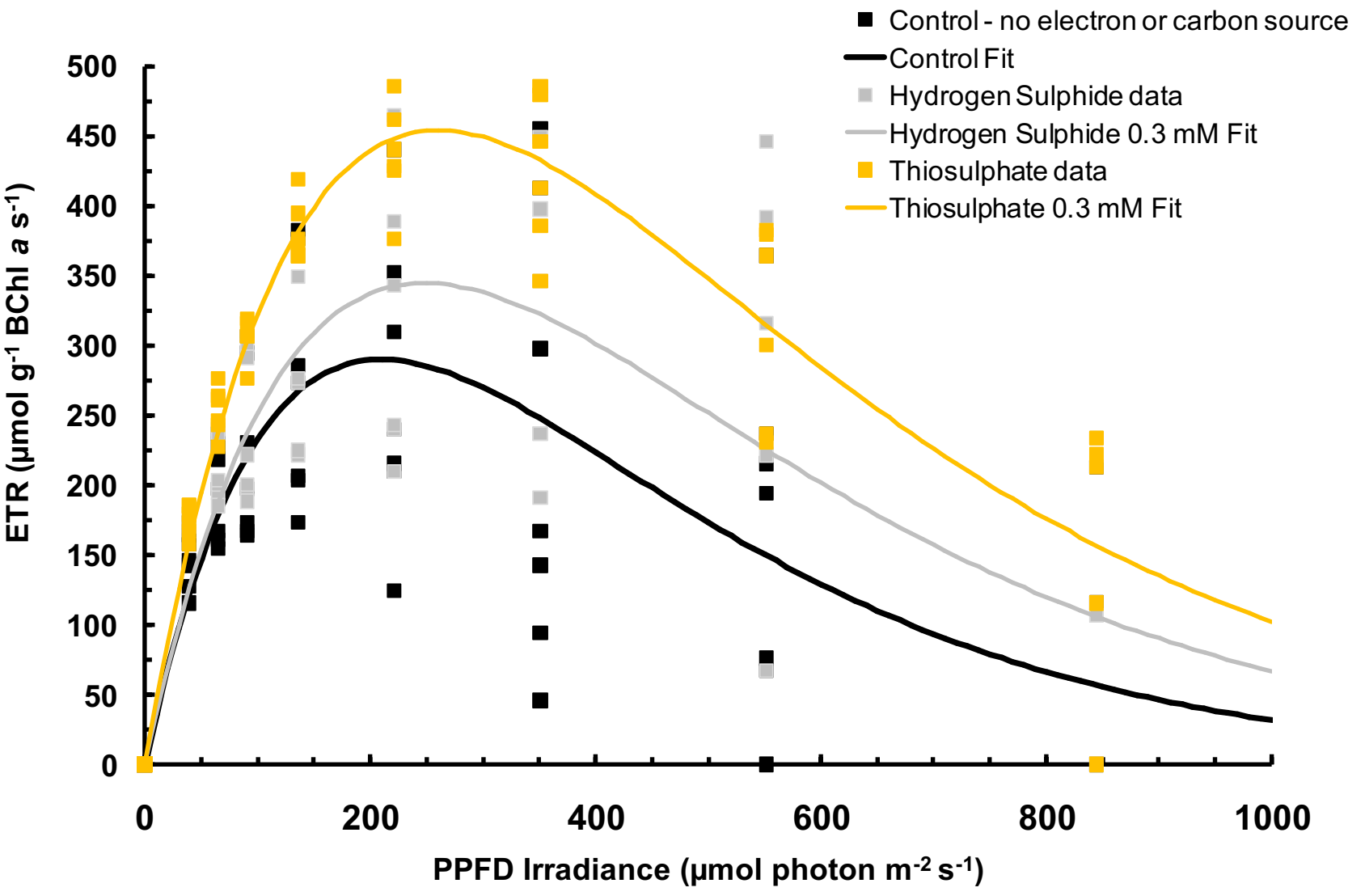

Fig. 5 Photosynthetic electron transport rate of *Rhodobacter* incubated in electron source-free PM medium (control) and supplied with Na_2S or thiosulphate (300 mmol m^{-3}). H_2S marginally increased the photosynthetic ETR and so was being used as an electron source for photosynthesis. Thiosulphate stimulated ETR by more than 50% and so was being used as an electron source. Similar experiments using Fe(II) as a potential e^- source showed that *Rhodobacter* could not use Fe(II) as an electron source unlike most PS Bacteria (Refs 1,2,3)

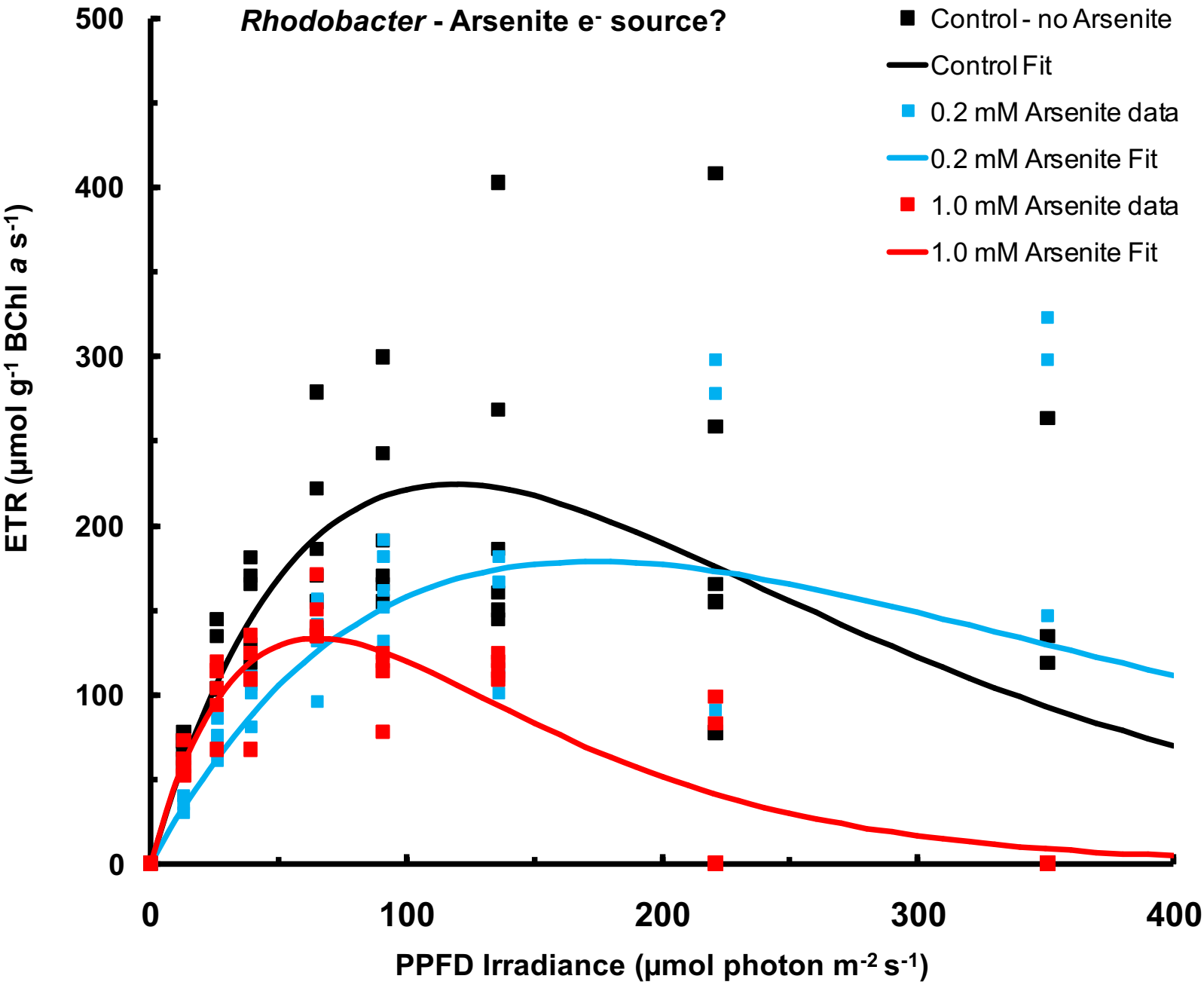

Fig. 6 Photosynthetic electron transport rate of *Rhodobacter* incubated in electron source-free PM medium (control) and supplied with 0.2 mol m^{-3} As(III) and 1 mol m^{-3} As(III). As(III) did not increase the photosynthetic ETR and so As(III) could not be used as an electron source for photosynthesis (cf. Ref 5)

Footnotes

Conclusions

• Arsenite (As(III)) is toxic but Arsenate (As(V)) has no short-term inhibitory effects. Response to As(V) is different under high P compared to low-P conditions.

• $Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.

• As(III) does not act as an electron source for photosynthesis in *Rhodobacter*.

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(5) Kulp, T.R. et al., (2008.) Arsenic(III) fuels anoxygenic photosynthesis in hot spring biofilms from Mono Lake, California. Science **321,** 967 – 970, doi:10.1126/science.1160799

How to Construct the Poster

Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia aarhiza*.

Raymond J. RITCHIE[1] and Siriporn NAKPHET[1]

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Summary

An arsenic resistant nitrogen fixing photosynthetic bacterium found to live inside *Wolffia aarhiza* plants has been cultured and identified as a *Rhodopseudomonad* species, most likely a strain of *Rhodobacter capsulatus*. It has BChl *a* as its primary photosynthetic pigment and has spectral properties typical of a Rhodopseudomonad. Blue-diode-based PAM (Pulse Amplitude Modulation) technology can be used to measure the photosynthetic electron transport rate (ETR) of the organism. The absorptance of the *Rhodobacter* films on glass fibre discs was measured and used to calculate actual ETR as mol e^- g^{-1} BChl *a* s^{-1}. ETR *vs.* Irradiance (E) curves fitted the waiting-in-line model ($ETR = (ETR_{max} \times E/E_{opt}) \times \exp(1-E/E_{opt})$). Yield (Y) was only ≈ 0.3 to 0.4. *Rhodobacter* saturates at about 250 to 350 µmol photons m^{-2} s^{-1} or ≈ 15% sunlight and shows photoinhibition at high irradiances (overall E_{opt} was 298 ± 7.36 µmol quanta m^{-2} s^{-1}; ETR_{max} = 642 ± 10.6 µmol e^- g^{-1} BChl *a* s^{-1}; Alpha (α) = 6.05 ± 0.200 e^- $photon^{-1}$ m^2 g^{-1} BChl *a*). Photosynthetic performance was much worse in Low-P medium (n = 108, overall E_{opt} 158± 15.4 µmol quanta m^{-2} s^{-1}; ETR_{max} = 194 ± 13.5 µmol e^- g^{-1} BChl *a* s^{-1}; α = 3.30 ± 0.400 e^- $photon^{-1}$ m^2 g^{-1} BChl *a*). *Rhodobacter* is resistant to As (V) toxicity up to at least 1 mol m^{-3} in high and low P medium. The Ki for As(III) in High and Low-P are not significantly different: overall mean was 497 ± 100 mmol m^{-3} but there is a threshold effect below about 200 mmol m^{-3} As(III). Fe(II) and As(III) did not appear to act as electron sources but thiosulphate did act as an electron source for photosynthesis. $Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.

Keywords: Photosynthetic bacteria, *Rhodobacter*, anoxygenic photosynthesis, integrating sphere spectrophotometry, PAM fluorometry.

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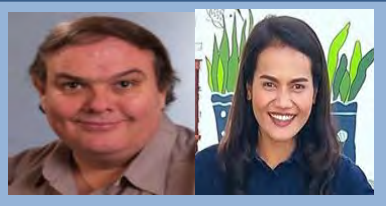
Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia aarhiza*.



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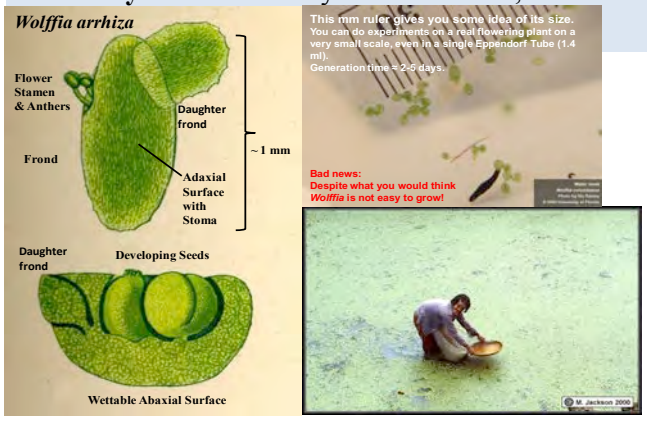

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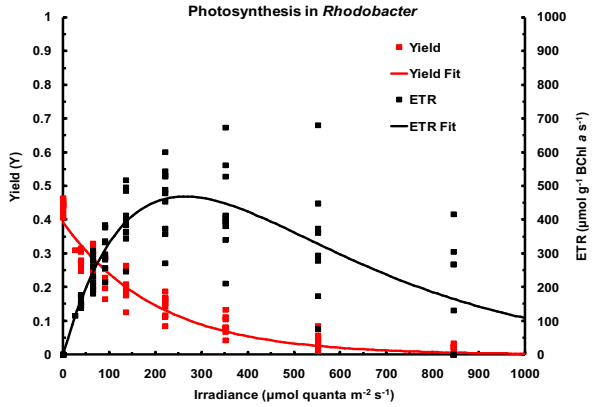

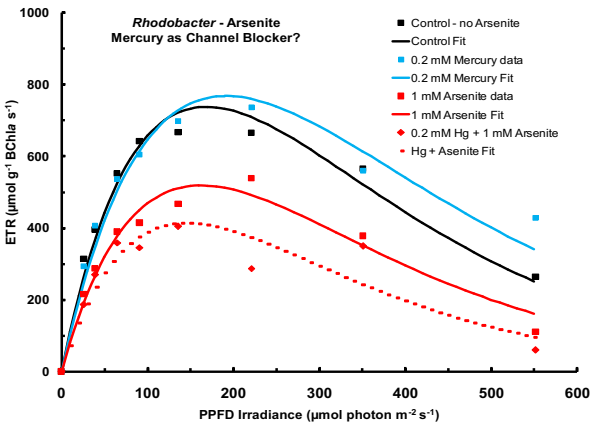

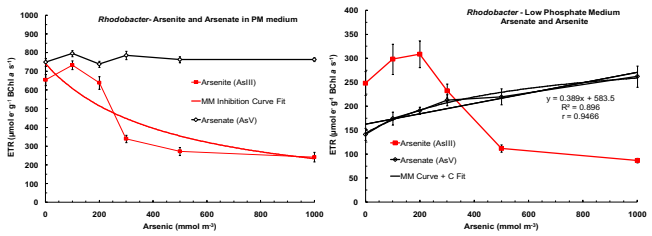

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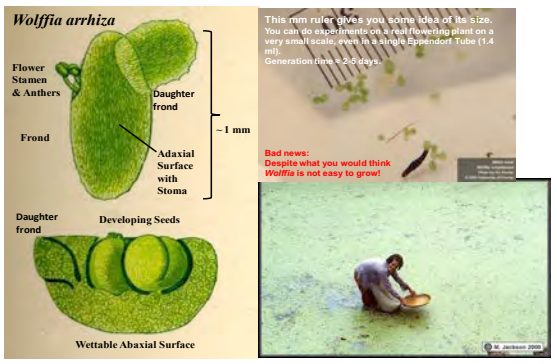

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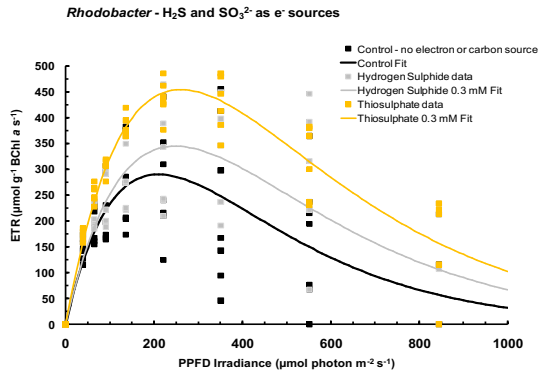

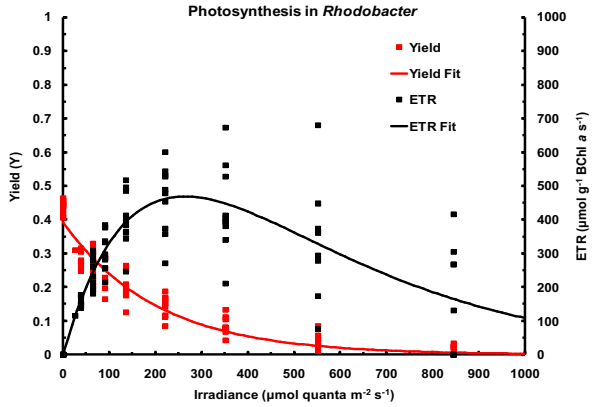

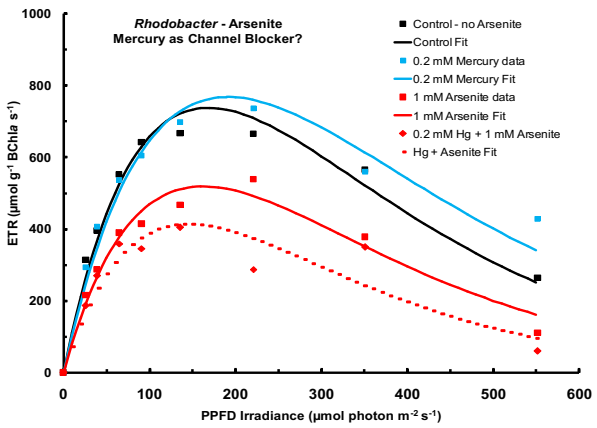

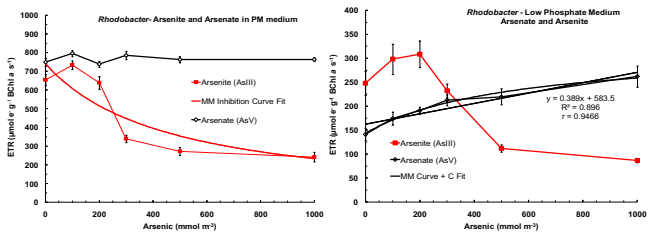

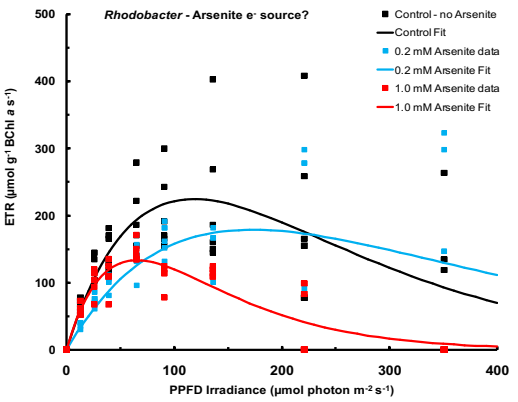

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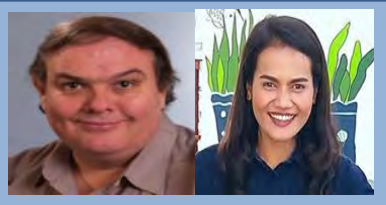

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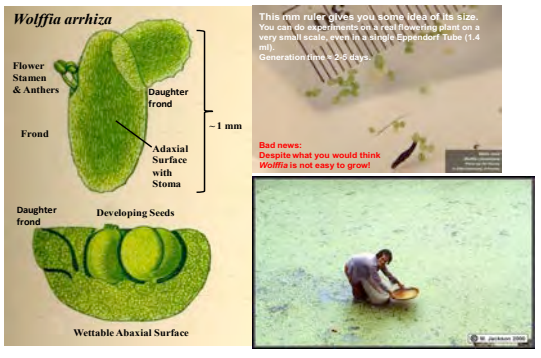

Fig. 1 *Wolffia* is a genus of very simple flowering plants (Angiosperms). They are the smallest known flowering plants. The very simple anatomy of *Wolffia* helps in the analysis and interpretation of arsenic uptake studies. No roots, stems or leaves or xylem and phloem. The very small size is also an advantage in the laboratory.
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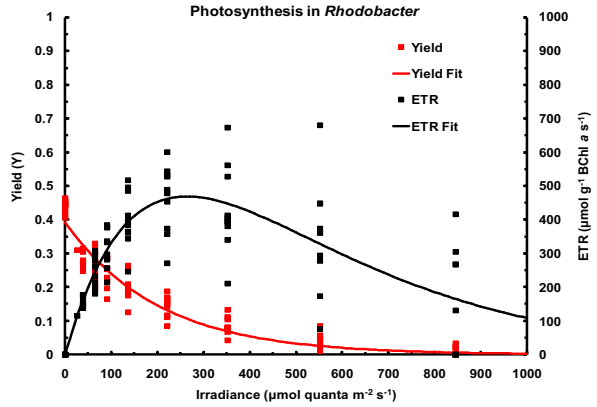

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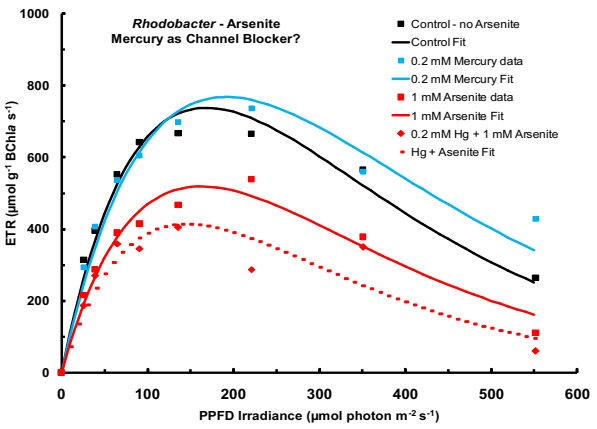

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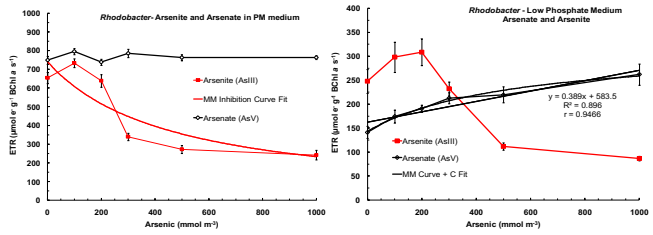

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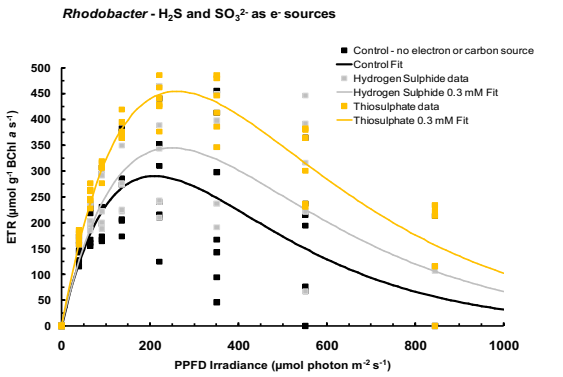

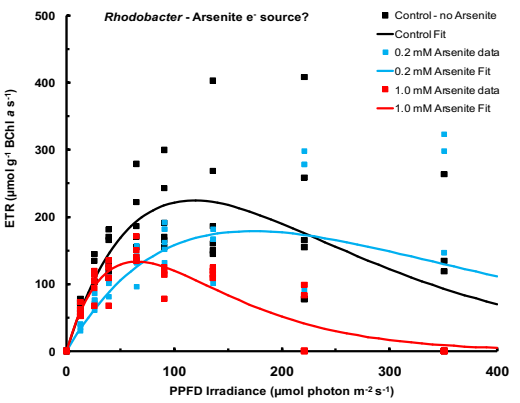

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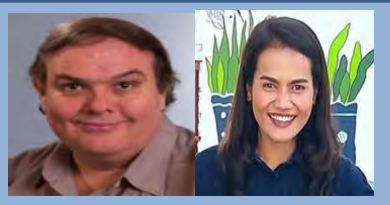

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Put in Fig 5 & 6 captions. Use the align tool.

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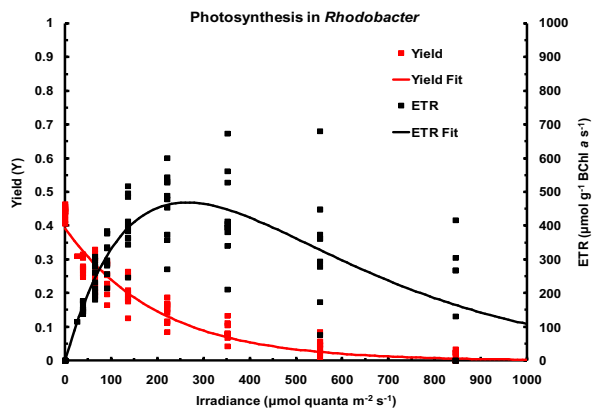

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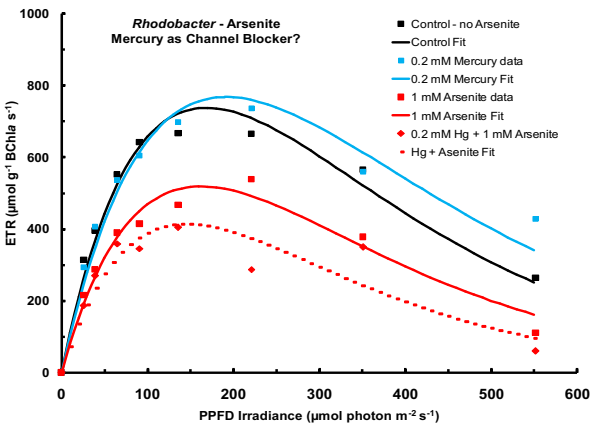

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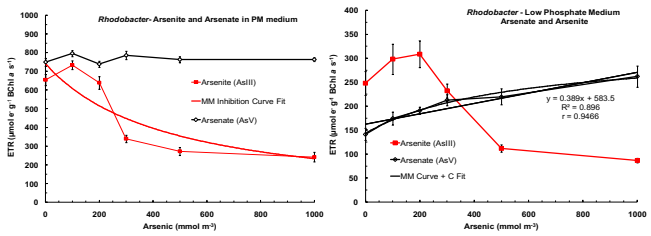

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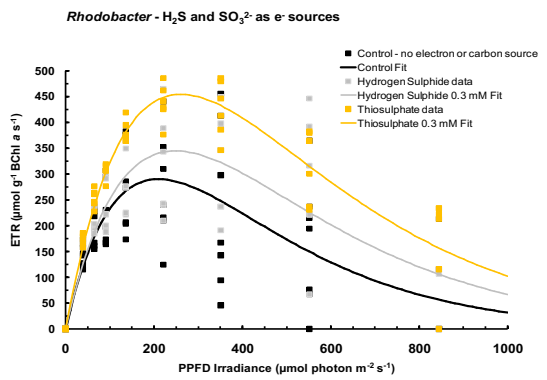

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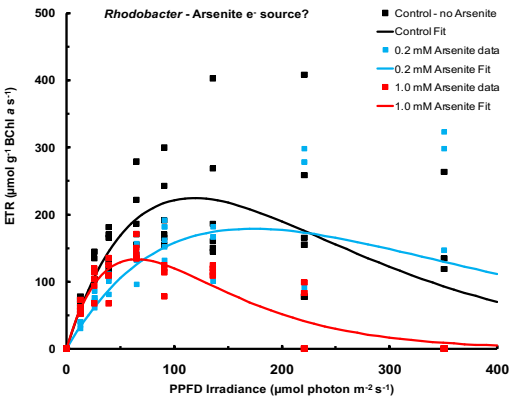

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Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia aarhiza*.

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Corresponding author: Raymond J. RITCHIE, E-mail: raymond.ritchie@uni.sydney.edu.au, raymond.r@phuket.psu.ac.th

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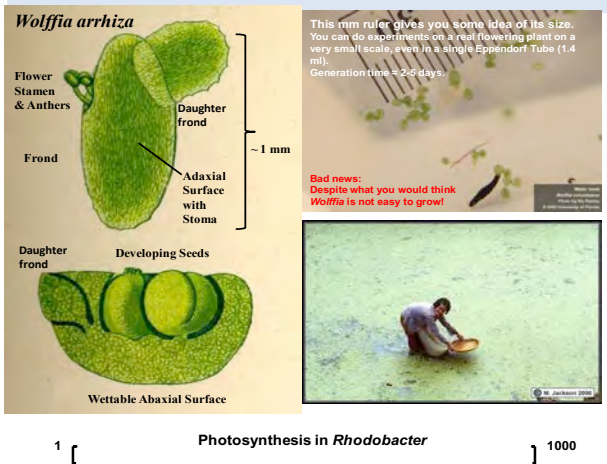

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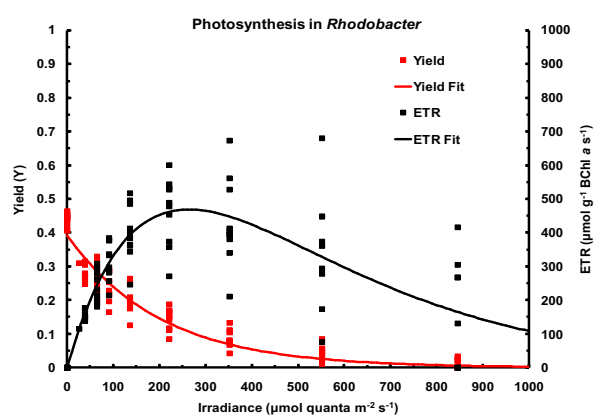

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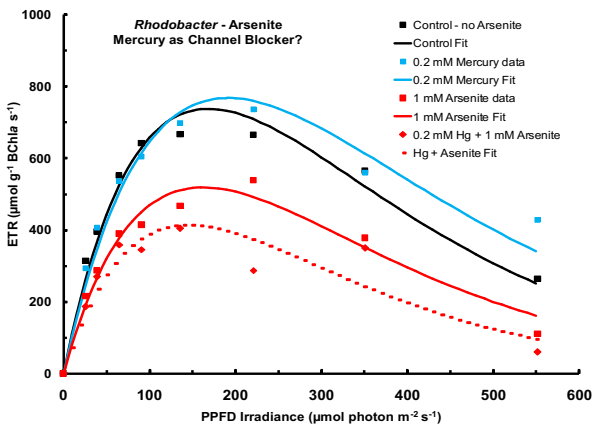

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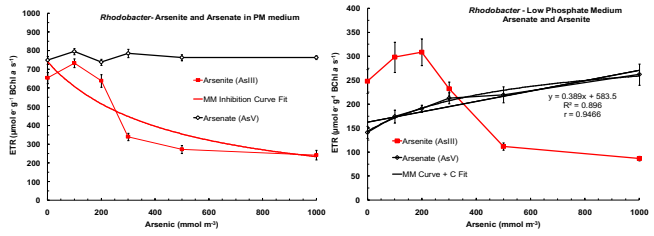

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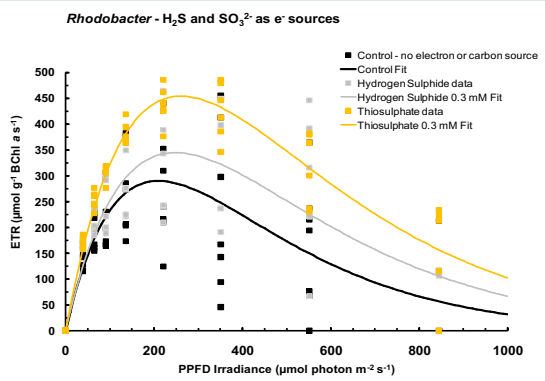

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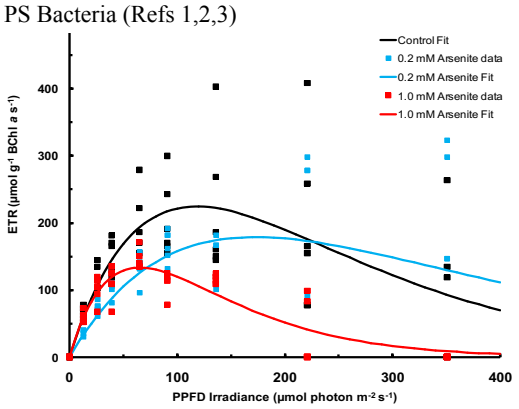

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References

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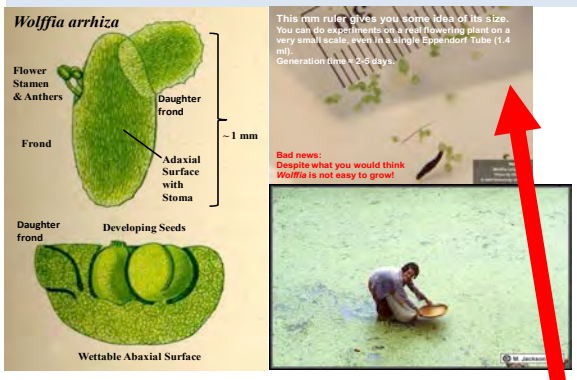
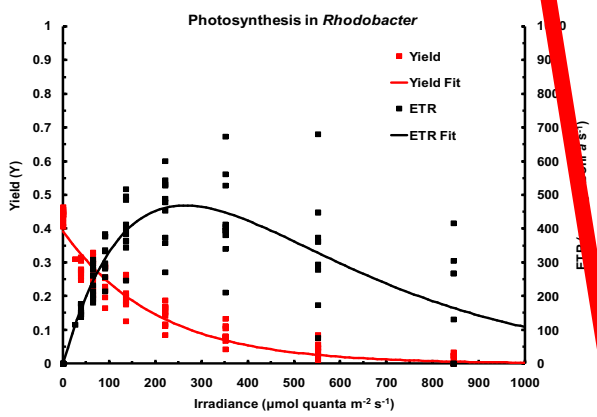
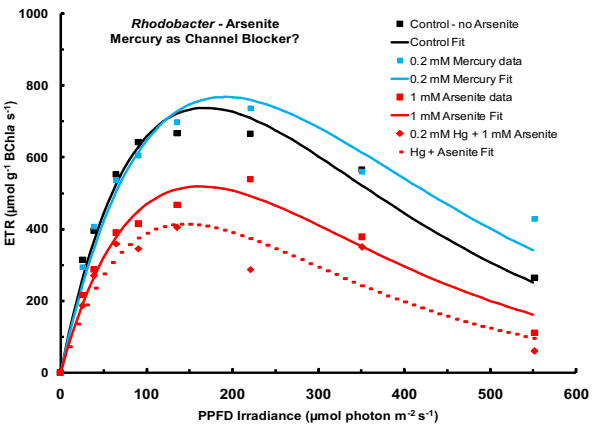
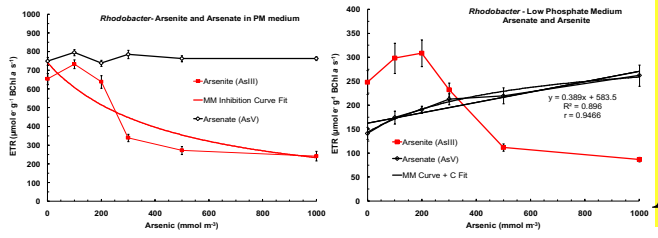


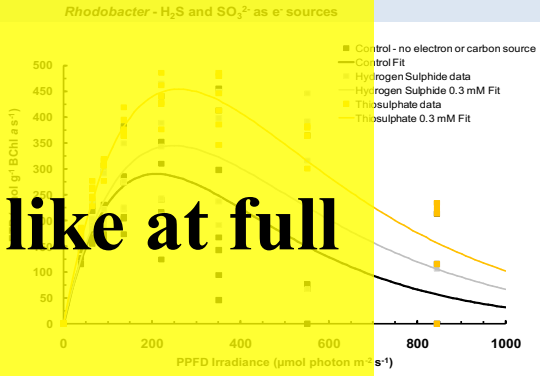


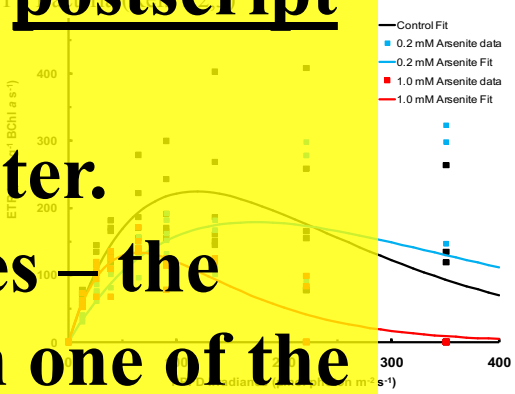




Final Steps

- Check what it looks like at full screen (A1 Size).
- Can you read everything from 1.5 m away?
- Any post-script file problems?
- Make sure you use a postscript font.
- Group the entire poster.
- Anything wrong? Yes – the white and red type on one of the pictures does not show very well. That was a mistake.
- Make a pdf of the *grouped poster* and get the pdf printed..





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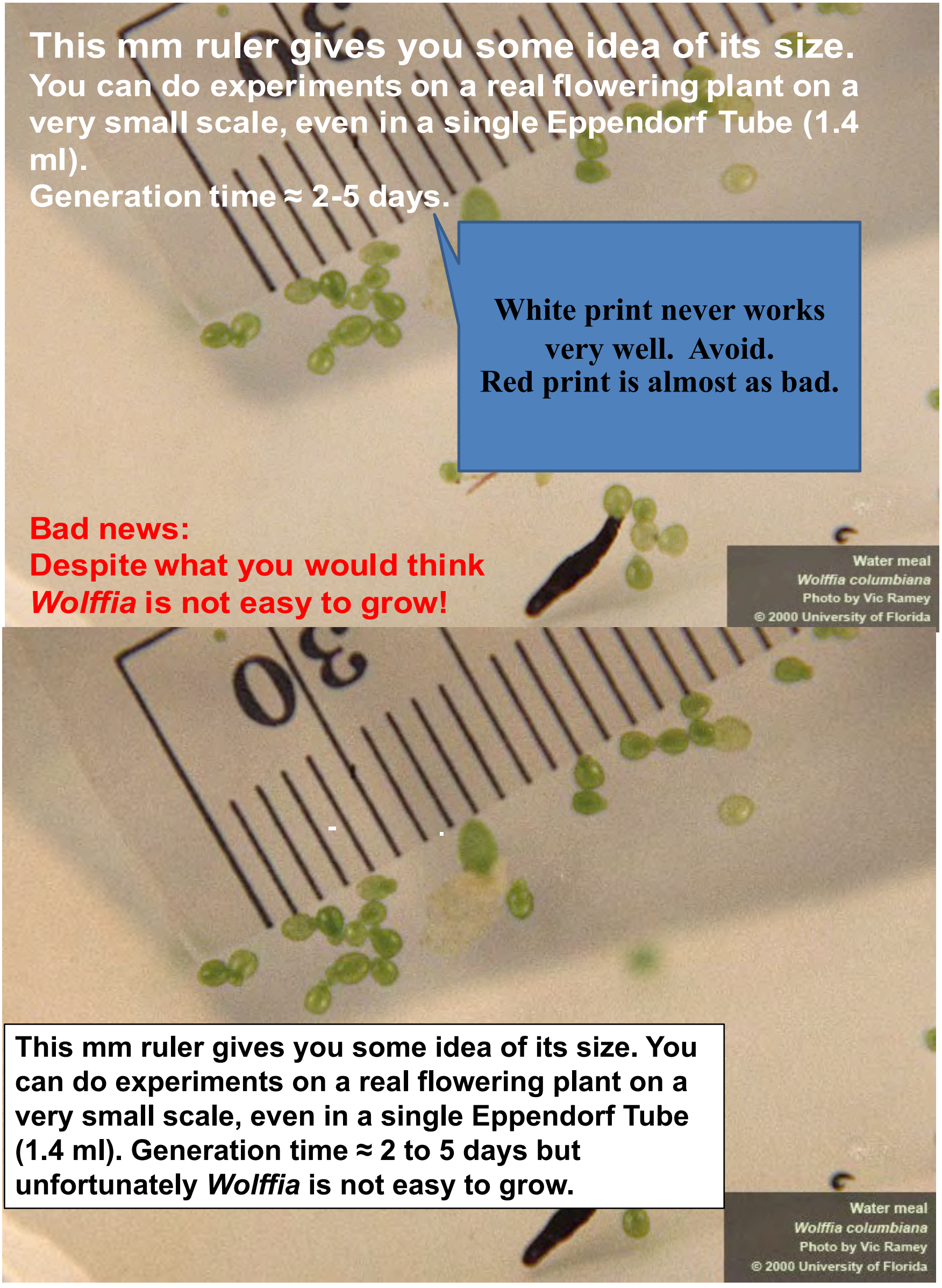

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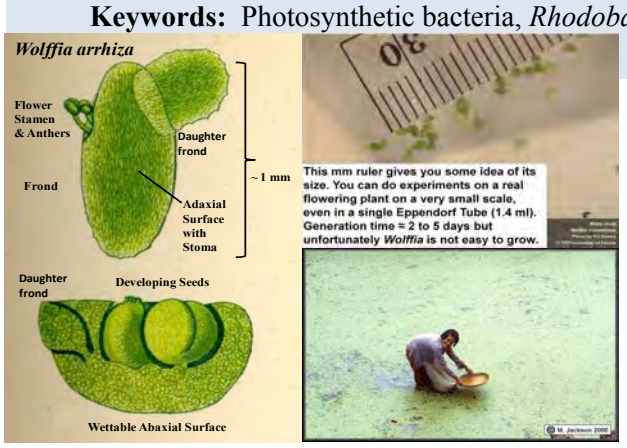
An arsenic resistant nitrogen fixing photosynthetic bacterium found to live inside *Wolffia aarhiza* plants has been cultured and identified as a *Rhodopseudomonad* species, most likely a strain of *Rhodobacter capsulatus*. It has BChl *a* as its primary photosynthetic pigment and has spectral properties typical of a Rhodopseudomonad. Blue-diode-based PAM (Pulse Amplitude Modulation) technology can be used to measure the photosynthetic electron transport rate (ETR) of the organism. The absorptance of the *Rhodobacter* films on glass fibre discs was measured and used to calculate actual ETR as mol e^- g^{-1} BChl *a* s^{-1}. ETR *vs.* Irradiance (E) curves fitted the waiting-in-line model ($ETR = (ETR_{max} \times E/E_{opt}) \times \exp(1-E/E_{opt})$). Yield (Y) was only ≈ 0.3 to 0.4. *Rhodobacter* saturates at about 250 to 350 µmol photons m^{-2} s^{-1} or ≈ 15% sunlight and shows photoinhibition at high irradiances (overall E_{opt} was 298 ± 7.36 µmol quanta m^{-2} s^{-1}; ETR_{max} = 642 ± 10.6 µmol e^- g^{-1} BChl *a* s^{-1}; Alpha (α) = 6.05 ± 0.200 e^- photon^{-1} m^2 g^{-1} BChl *a*). Photosynthetic performance was much worse in Low-P medium (n = 108, overall E_{opt} 158± 15.4 µmol quanta m^{-2} s^{-1}; ETR_{max} = 194 ± 13.5 µmol e^- g^{-1} BChl *a* s^{-1}; α = 3.30 ± 0.400 e^- photon^{-1} m^2 g^{-1} BChl *a*). *Rhodobacter* is resistant to As (V) toxicity up to at least 1 mol m^{-3} in high and low P medium. The Ki for As(III) in High and Low-P are not significantly different: overall mean was 497 ± 100 mmol m^{-3} but there is a threshold effect below about 200 mmol m^{-3} As(III). Fe(II) and As(III) did not appear to act as electron sources but thiosulphate did act as an electron source for photosynthesis. $Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.

Keywords: Photosynthetic bacteria, *Rhodobacter*, anoxygenic photosynthesis, integrating sphere spectrophotometry, PAM fluorometry.



Final Steps

- **Fix text**

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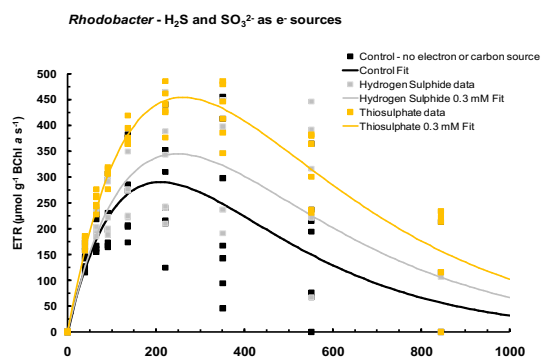

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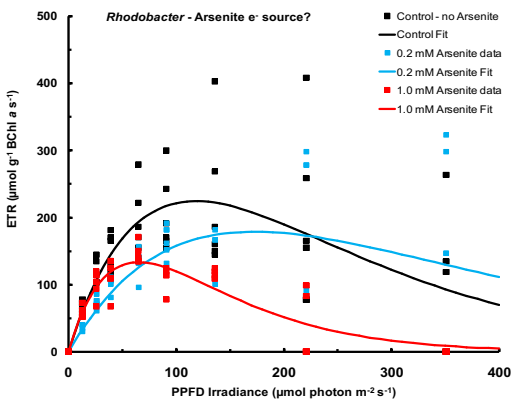

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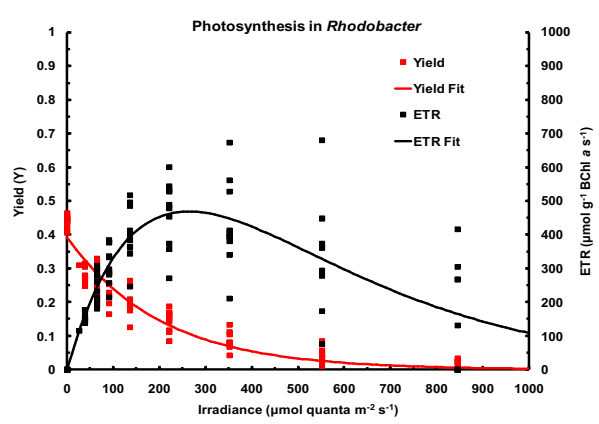

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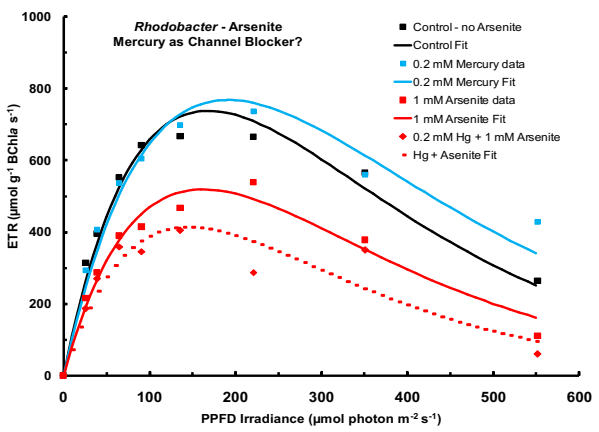

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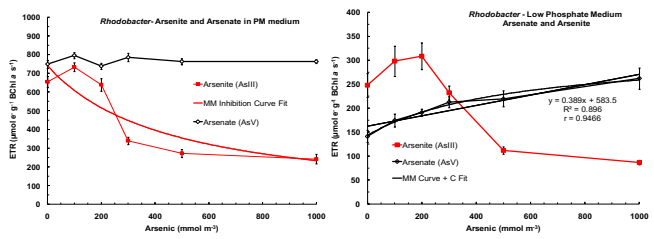

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Final Step

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Conclusions

- **Arsenite (As(III)) is toxic but Arsenate (As(V)) has no short-term inhibitory effects. Response to As(V) is different under high P compared to low-P conditions.**
- **$Al(OH)_3$ and Hg^{2+} are common aquaporin channel blocking agents but neither acted as protectants against As(III) toxicity.**
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References

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Arsenic toxicity in a Photosynthetic Bacterial Symbiont of *Wolffia aarhiza*.

Raymond J. RITCHIE[1] and Siriporn NAKPHET[1]

[1]Technology and Environment, Prince of Songkla University-Phuket, Kathu, 83120, Thailand.
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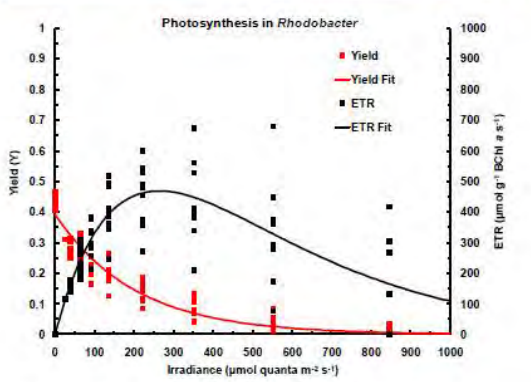

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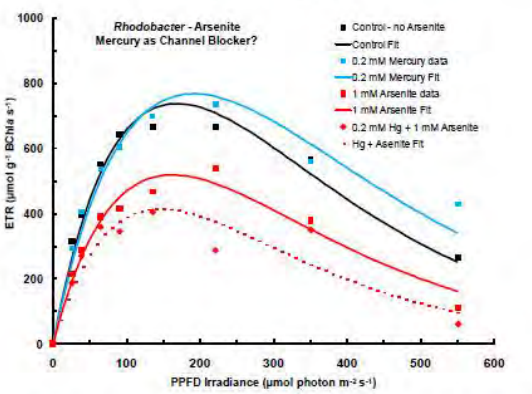

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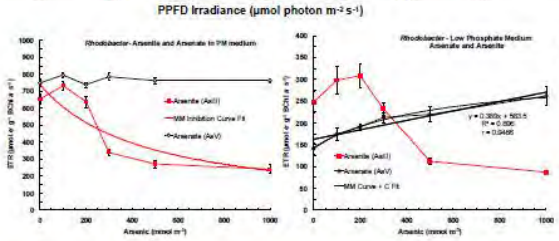

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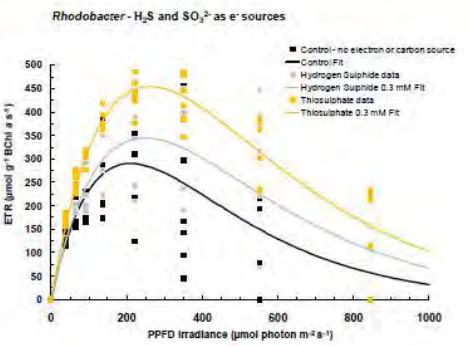

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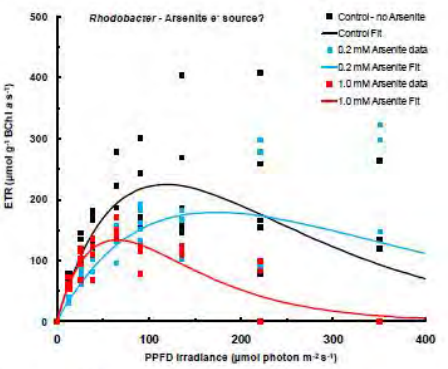

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