



Studying the effects of leaf gas film using microsensors

Flooding of vegetation introduces a number of challenges to the plants. Reduced oxygen solubility combined with slower diffusion of gases restricts photosynthesis under water as O₂ and CO₂ exchange between the plant and the environment is limited. Some plants have developed smart ways to survive flooding. The referred two articles focus on the function of leaf gas film formed on the superhydrophobic leaf.

There has been suggestions of improved CO₂ uptake during submergence of plants with leaf gas films; however, the function of leaf gas films is not well-understood. In Pedersen et al. (2009) this function is investigated by setting up laboratory experiments and in Winkel et al. (2013) the studies are repeated in paddy field rice in the Philippines. This research summary will focus on the data the researchers obtained using microsensors in laboratory and in situ experiments.

Laboratory Setup

The dynamics of root pO₂ in light and darkness were investigated using O₂ microsensors. Four-week-old plants (*Oryza sativa* L.) were kept in a chamber that allowed for separate medium for root and shoot of the plant. The roots were incubated in deoxygenated medium, whereas the shoots were incubated in a medium containing 200 mmol m⁻³ free CO₂ and O₂ in air equilibrium. The chamber was covered with PVC foil to prevent possible contact between leaves and air. This setup mimicked in situ conditions and allowed for studying the internal aeration of the plant.



Laboratory setup with double chamber, the sensor is positioned into root cortex. Photo: Ole Pedersen

A. Winkel, T. D. Colmer, A. M. Ismail, and O. Pedersen
Internal aeration of paddy field rice (*Oryza sativa*) during complete submergence - importance of light and floodwater O₂. *New Phytol* 197 (4):1193-1203, 2013.

O. Pedersen, S. M. Rich, and T. D. Colmer.
Surviving floods: leaf gas films improve O₂ and CO₂ exchange, root aeration, and growth of completely submerged rice. *The Plant Journal* 58 (1):147-156, 2009.

Unisense O₂ microsensors with a tip diameter of 25 μm were connected to a Unisense amplifier, mounted on a micromanipulator and positioned into the root cortex. The effect of leaf gas film was studied by measuring root pO₂ before and after brushing the leaves with a diluted Triton-X-100 solution, to remove the gas film.

Field Setup

Root pO₂ of paddy field rice was measured during two days of complete submergence. Four-week-old rice plants were planted into a paddy field. Roots were exposed and the microsensors were placed 200 μm into the root tissue. Hereafter both root and microsensor were covered with soil, placing the sensor tip approx. 4 cm below soil surface. To investigate the function of the leaf gas film, leaves of selected plants had their gas film removed 3 hours prior to flooding. Light status was monitored by a weather station placed approx. 440 m from the paddy field.



Brushing leaves with a diluted Triton-X-100 solution to remove leaf gas film. Photo: Ole Pedersen



O₂

N₂O

H₂S

NO

H₂

pH

Redox

Temp

EP

Conclusion on lab and field data

Leaf gas films are hypothesized to improve internal aeration of the plant during the day, as leaf gas films enhance CO₂ uptake and thereby promote photosynthesis. This is seen as higher root pO₂ in plants with intact gas film compared to plants without. The two studies described in this flyer investigate gas film based on laboratory experiments and experiments in the field.

In the laboratory, root pO₂ increase in light periods in submerged plants, probably due to reduced outward diffusion of photosynthetically produced O₂. Removal of gas film resulted in a decrease in root pO₂ to just below the initial level (see fig 1A). This can be explained by decreased O₂ production by photosynthesis as a result of impeded CO₂ entry. In darkness, root pO₂ rapidly decreased to 25% (see fig. 1B) of when submerged in light and declined to close to zero when the gas film was removed.

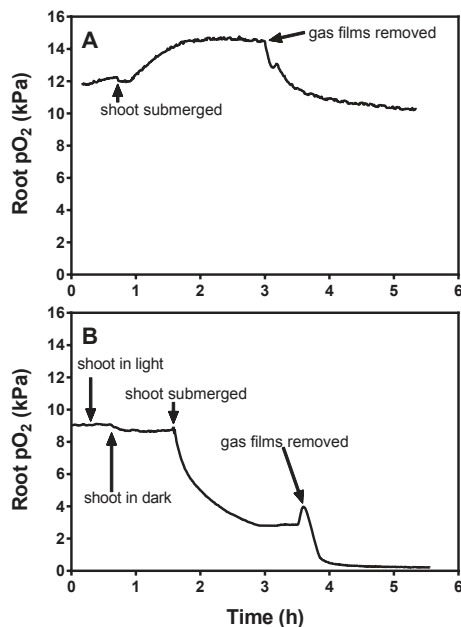


Fig. 1 Root pO₂ measured in light (A) or darkness (B) before and after submerge and with or without removal of gas film. Figure adapted from Pedersen et al 2009.

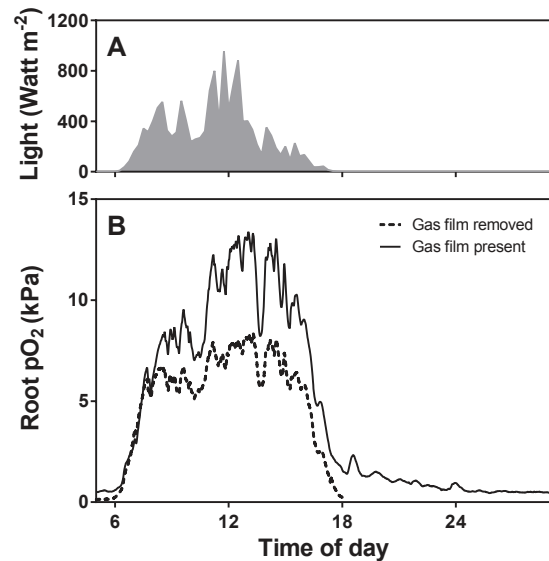


Fig. 2. (A) surface light was measured approx. 440 m from field location using a weather station. (B) Root pO₂ measured in completely submerged plants with or without gas film. Adapted from Winkel et al. 2013.

In the field, plants with intact leaf gas films had higher daytime root pO₂ compared to plants without leaf gas film during the first day of measurements (fig. 2B), supporting laboratory data. The difference in root pO₂ of plants with or without leaf gas film was, however, not significant on the second day (data not shown), suggesting that the leaf gas film had either been reestablished or that emerging new leaves with intact gas film had masked the effect of the gas film removal on the leaves from the day before.

Although data obtained in the field were only significant on the first of day of measurement, the in situ data support the findings obtained in the laboratory experiments by showing a positive correlation between leaf gas film and increased root pO₂. Combining laboratory and in situ measurements thus contributed to understanding how leaf gas films help the rice plant survive flooding.

Recommended Unisense Products

	LABORATORY PRODUCTS	FIELD PRODUCTS
SENSOR	O ₂ , H ₂ S, H ₂ , NO, N ₂ O, pH, Redox Temperature Oxygen MicroOptode	O ₂ , H ₂ S, H ₂ , NO, N ₂ O, pH, Redox Temperature Oxygen MicroOptode
AMPLIFIER	fx-6 UniAmp Opto-F1/Opto-F4 UniAmp	Field Microsensor Multimeter UnderWater Meter
SYSTEMS	MicroProfiling System MicroRespiration System	Field MicroProfiling System MiniProfiler MP4/8 Eddy Correlation System
SOFTWARE	SensorTrace Suite	SensorTrace Suite