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Leaf Wand for Measuring Chlorophyll Fluorescence on Cylindrical Leaves and its Application on *Juncus roemerianus* (Black Needlerush)

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ABSTRACT

Chlorophyll fluorescence is a well established technique to rapidly and non-invasively determine photosynthesis parameters in plant leaves. It can be used in both laboratory and field settings, and frequently dark-adaptation of a leaf sample is called for. In the field, this can be accomplished on flat leaves using standard leaf clips supplied by instrument manufacturers. However, not all plant leaves are flat, many are cylindrical or otherwise three-dimensional in shape. The standard leaf clip does not close fully on three-dimensional leaves, therefore, does not allow the sample to be properly dark adapted in the field. A new leaf "wand" was developed that can be slipped over an entire cylindrical leaf or culm of rushes and sedges for both light- and dark-adapted measurements. This new leaf wand is compared to the standard leaf clip (DLC-8) using a Walz mini-PAM on Juncus roemerianus (Black needlerush). Results indicate that dark-adapted yield measurements are not significantly different between leaf clips, while light-adapted yields are higher with the leaf wand. The potential sources of difference in the optical path of the excitation light and fluorescence return are discussed and compared between leaf clips. Construction of specialized leaf wands should be considered for any leaves are not flat and therefore that do not fit the standard leaf clip for complete dark-adaptation under field conditions.

***Keywords*:** *Juncus roemerianus*, chlorophyll fluorescence, leaf clip, cylindrical leaf, optical path length

1. Introduction

Chlorophyll fluorescence is a tool to measure photophysiological processes in vivo and this technique has been used successfully to demonstrate physiological stress in a wide variety of plant species [1-4]. Various instruments have been designed and are commercially available to researchers [5], including portable instruments for field-application, such as the Walz Mini-PAM (Walz GmBH). The availability of reliable instrumentation has led to chlorophyll fluorescence becoming a widely adopted, robust and reliable field technique that is easy to carry out, non-destructive, and rapid [6].

Chlorophyll fluorescence of photosystem II (PSII) can be measured by a variety of techniques including the pulse amplitude modulated (PAM) technique. Chlorophyll fluorescence can provide an instantaneous measure of the effective quantum yield (ΔF/Fm') of PS II under prevailing ambient light conditions [7]. Alternatively, more standardized differences among leaves can also be determined by measuring the potential quantum yield (Fv/Fm) of dark-adapted samples. A healthy plant absorbs light from the sun, and directs a proportion of the solar energy absorbed into photosynthesis. Generally, the maximum possible proportion of the solar energy absorbed into photosynthesis is around 83%, equivalent to a quantum yield of 0.830 [6]. As plants become stressed, reductions in the quantum yield of photosynthesis are evident, which can be used as a rapid screening technique [8].

Leaf samples can be dark adapted with flat leaf clips that are supplied with the instrument. These are attached to a flat plant leaf and serve to occlude a small area of the leaf. After a pre-determined period of time, a shutter built into the clip is manually opened, exposing the leaf area under the clip to very low intensity red light transmitted through fiber-optics in the case of the Walz PAM. The chlorophyll in the dark-adapted area of the leaf fluoresces and the initial fluorescence (Fo) is recorded. Upon illumination with a high intensity burst of saturating actinic light through the fiber-optics, the pigments associated with PSII become overwhelmed and the maximal fluorescence (Fm) is recorded. The difference between the maximal and initial fluorescence levels (Fm-Fo) is called the variable fluorescence (Fv) and from this the ratio Fv/Fm, or potential quantum yield, is calculated. In an analogous fashion the effective quan-

