Effects of winter stress on photosynthetic electron transport and energy distribution between the two photosystems of pine as assayed by chlorophyll fluorescence kinetics

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Abstract. The fluorescence kinetics of both intact needles and isolated chloroplasts of summer active and winter stressed Pinus sylvestris were measured at both room temperature and 77 K. It was confirmed that winter stress inhibited the photochemical capacity of photosystem II but also that winter stress caused the strongest inhibition of the electron transport at the site where the plastoquinone pool is reduced. Parallel analyses of the fluorescence characteristics of photosystem II (F693) and photosystem I (F735) during photosystem II trap closure furthermore revealed that the yield of spill-over of excitation energy from photosystem II to photosystem I decreased upon winter stress. We suggest that this is because of an increased radiationless decay of excitation energy both at the reaction center and antennae levels of photosystem II. There is, however, also a possibility that the decreased yield of spill-over is accentuated by a partial detachment of the light harvesting chlorophyll a/b complex from photosystem II upon winter stress.

Abbreviations
DCMU, 3, (3, 4-dichlorophenyl-1, 1-demethylurea); DGDG, digalactosyldi-glyceride; Fm, fluorescence when the reaction centers of PS II are closed; Fo, fluorescence when the reaction centers of PS II are open; Fv = Fm minus Fo; LHCP, light harvesting chlorophyll protein; MGDG, monogalactosyldi-glyceride.

Introduction
It is well recognized that exposure of evergreen conifers to sub-freezing winter temperatures causes a more or less complete inhibition of the potential for net photosynthesis [7, 18]. This inhibition is not primarily confined to a restricted CO2 diffusion through closed stomata [22] but it occurs at the chloroplast level [15]. Full recovery of the rate of net photosynthesis during the spring usually takes about one month [14, 18].

The effects of severe winter stress on the chloroplast structure and function is complex. At least three factors have to be considered: 1) low freezing temperature, itself; 2) freeze-desiccation of the cells; 3) light absorbed in excess by the chlorophyll antennae at low temperatures. The following major winter stress effects of pine chloroplasts have been observed; 1) changes
of chloroplast structure resulting in partial destacking [10]; 2) partial bleaching of chlorophyll and partial destruction of antennae [17]; 3) decreased amount and decreased level of fatty acid unsaturation of MGDG [14]; 4) partial inhibition of the two photoreactions with the main inhibition in the electron transport chain linking the two photosystems [14, 16].

The aim of this work is to evaluate the use of fluorescence of chlorophyll in pine needles and isolated chloroplasts to quantify inhibition induced by winter stress of photosystem II and in the electron transport chain linking the two photosystems. We also use low temperature fluorescence kinetics measurements for evaluating the effects of winter stress on the energy distribution within the photosynthetic apparatus using the bipartite model for the antennae organization of the two photosystems, as described by Butler and coworkers [3].

Materials and methods

Current year needles were collected from an about 20 year-old naturally grown tree of *Pinus sylvestris* (Umeå N63°50'E20°20') on April 15, May 31, June 1, 7 and 16 and July 25 and 28, 1983. This is the period of the year when the function of photosynthesis recovers from winter stress, with the most rapid reactivation in May [14]. The needles were used for analyses immediately, but the winter stressed needles harvested on April 15 were also stored in plastic bags in darkness at -18 °C for later control experiments. No effects on studied parameters were obtained by the storage.

Chloroplasts were isolated according to Martin et al. [8]. Chlorophyll was determined in 80% acetone [1].

Fluorescence kinetics of both intact needles and isolated chloroplasts were studied using a three-branched fiber optic based spectrofluorometer described elsewhere [12]. At room temperature the fluorescence intensity was read at 680 nm (half band width 6.4 nm) and at 77 K it was read at 693 (half band width 6.4 nm) and 729 nm (half band width 13 nm) originating from the reaction center chlorophyll a antennae of photosystem II and I, respectively [3]. The room temperature kinetics traces were recorded with a Gould 1040 two channel memory oscilloscope and the low temperature kinetics of the photosystem II and I fluorescences were recorded in parallel on a two pen chart recorder (Sekonic SS-250F). Excitation was with a broad band filter, 380–590 nm with a peak at 525 nm, at room temperature and at 477 nm (half band width 10 nm) at 77 K. The quantum flux densities of the actinic lights used in different experiments are given in the figure legends.

Results

A comparison of the kinetics of chlorophyll fluorescence at room temperature of whole needles of winter stressed (April 15) and summer active (July 15) pine was made (Figure 1). Very little variable fluorescence ($F_v = F_m$ minus