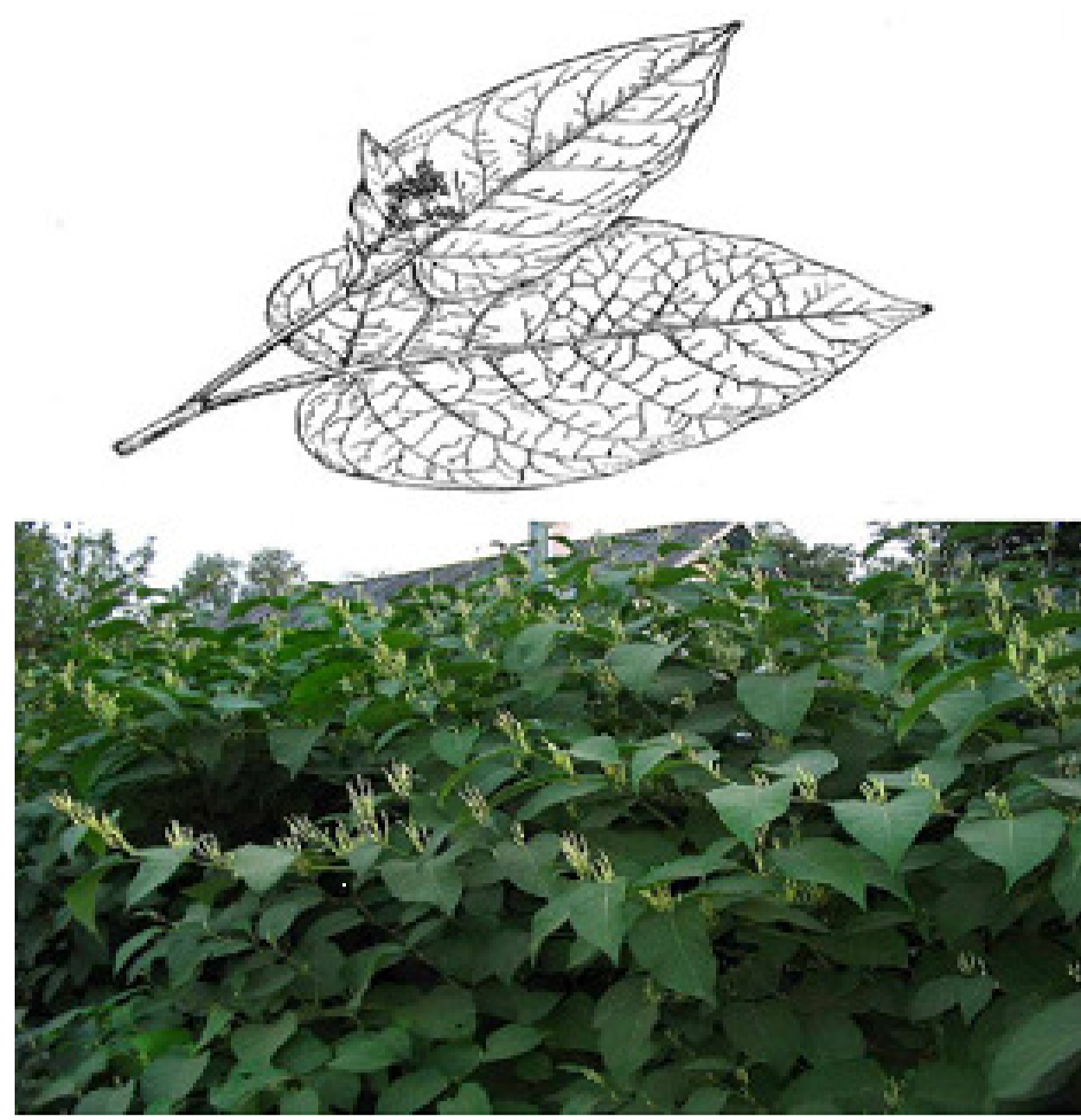


# SFC of Supercritical Fluid Extract of *Reynoutria Sachalinensis*

Glazunova S.A. (1), Pokrovskiy O.I. (2), Karavaev V.A. (1), Parenago O.O. (2)  
 1 M.V. Lomonosov Moscow State University, Faculty of Physics, Moscow, Russia  
 2 N.S. Kurnakov Institute of General and Inorganic Chemistry RAS, Moscow, Russia



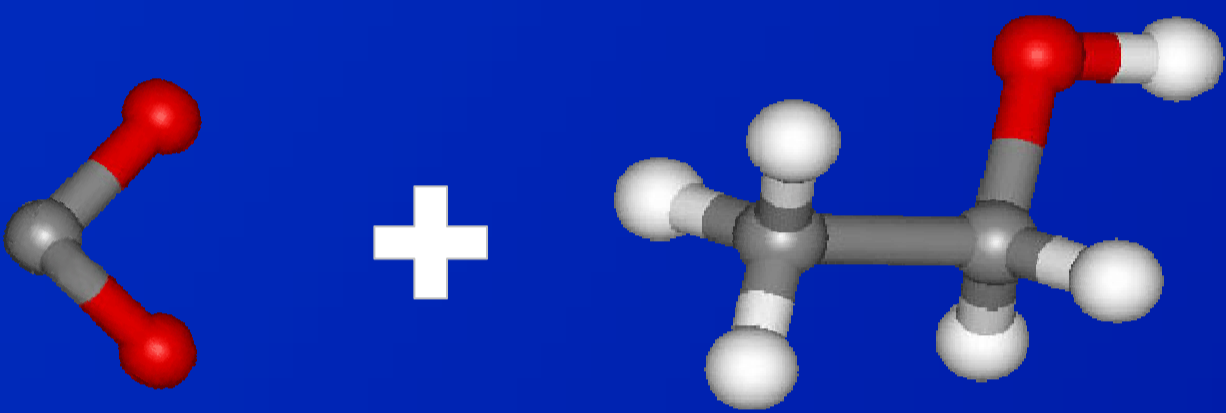
*Reynoutria sachalinensis*

New developing trend in ecology and agriculture is searching of natural fertilizers and fungicides that will be harmless to beneficial insects and animals. It is known that treatment of plants by water or ethanol extracts from giant knotweed, *Reynoutria sachalinensis* (F. Schmidt) Nakai, can protect plants from powdery mildew, induces resistance and is ministerial to increasing of photosynthetic activity. *Reynoutria sachalinensis* is usual plant for Japan and Far East of Russia, but it also grows in the central part of Russia. Ethanol extract of *Reynoutria sachalinensis* shows good results, but it is known now that ethanol can exert negative influence on plants in general and photosynthetic processes. And water extract of giant knotweed exerts very low positive influence, as this type of extraction allows to get a little amount of active compounds. It has occurred to us to try supercritical fluid extract of *Reynoutria sachalinensis*. Supercritical fluid extraction is known as a "green" technology. Supercritical fluid processing shows numerous advantages compared to traditional organic solvent extraction and water extraction. In traditional extraction, the residual solvent is unavoidable and is usually measured in parts per million (ppm). Also supercritical fluid extraction allows to get more percentage of desired active compound. We obtained three types of supercritical extracts of *Reynoutria sachalinensis* using the system SFE1000M1 produced by Thar Instruments Inc. This unit is situated in the Institute of General and Inorganic Chemistry, in demonstration center of the company ZAO "SCHAG".



SFE1000M1 by TharSFC

The difference in obtained extracts was in the eluent we used. For the first type of extracts we used carbon dioxide with ten percents of ethanol as solvent, for the second - carbon dioxide with two percents of ethanol and for the third one we used just carbon dioxide. For treatment of the plants the obtained supercritical fluid extracts were diluted with water to the final concentration of two percent.

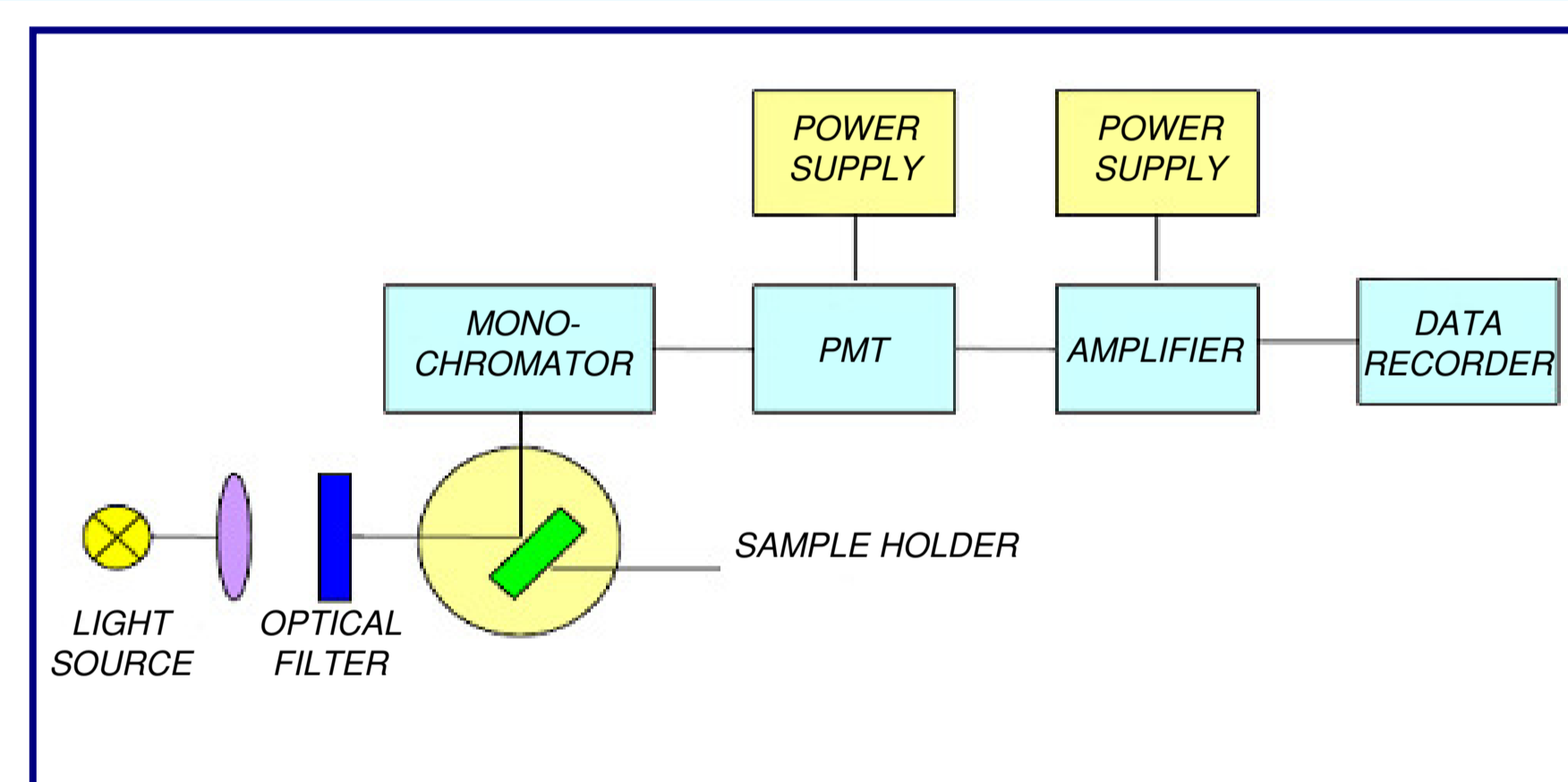


SF extract of *Reynoutria sachalinensis*



Bean leaves, treated with SF extract of *Reynoutria sachalinensis*

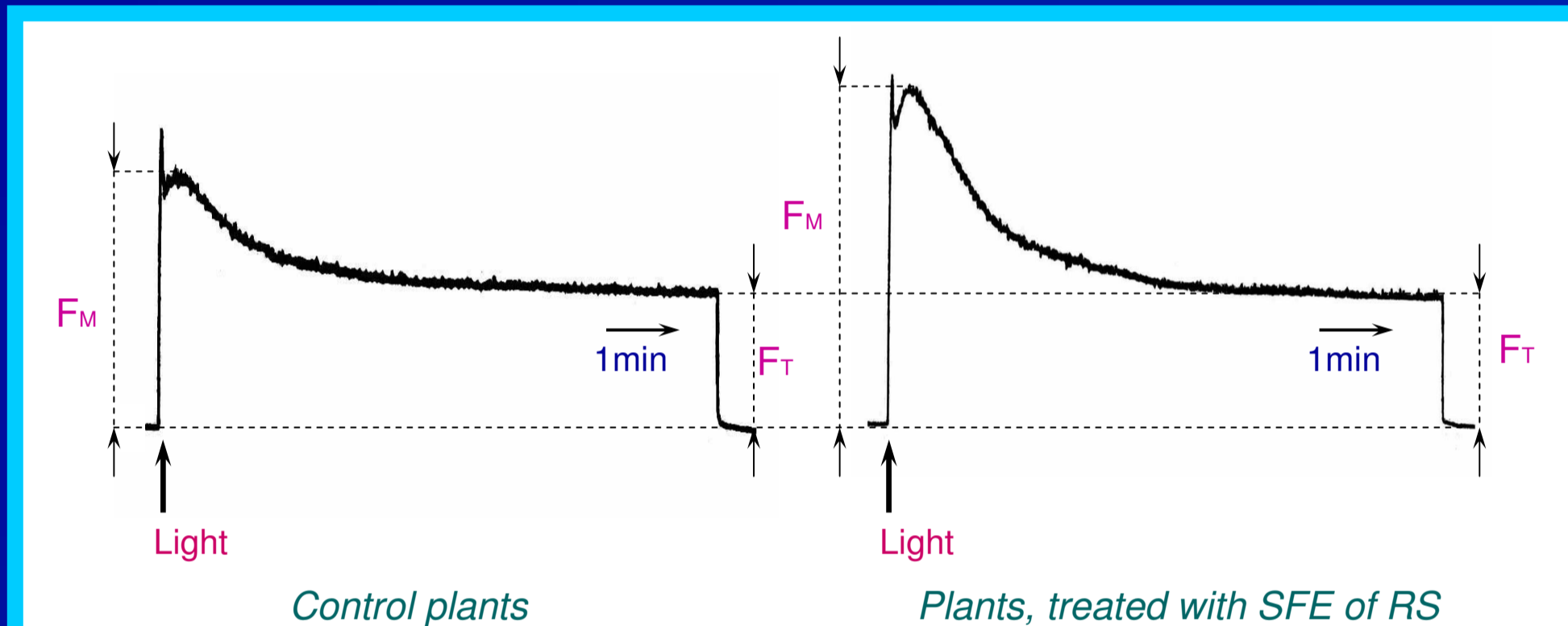
Bean leaves were sprayed with these solutions. We have studied the influence of treatment with the extracts on the photosynthetic activity of bean plants grown in the laboratory. The method used in the work is based on the registration of slow fluorescence induction (SFI) of the leaves allowing to monitor the photosynthetic processes *in situ*. Chlorophyll *a* fluorescence induction (FI) is now a widespread method used in photosynthesis research. This is because this method is non-invasive and highly sensitive, fast and easily measured, it requires relatively inexpensive equipment, and it contains important information about the photosynthetic apparatus.



Block scheme of the system for measuring slow fluorescence induction (SFI)

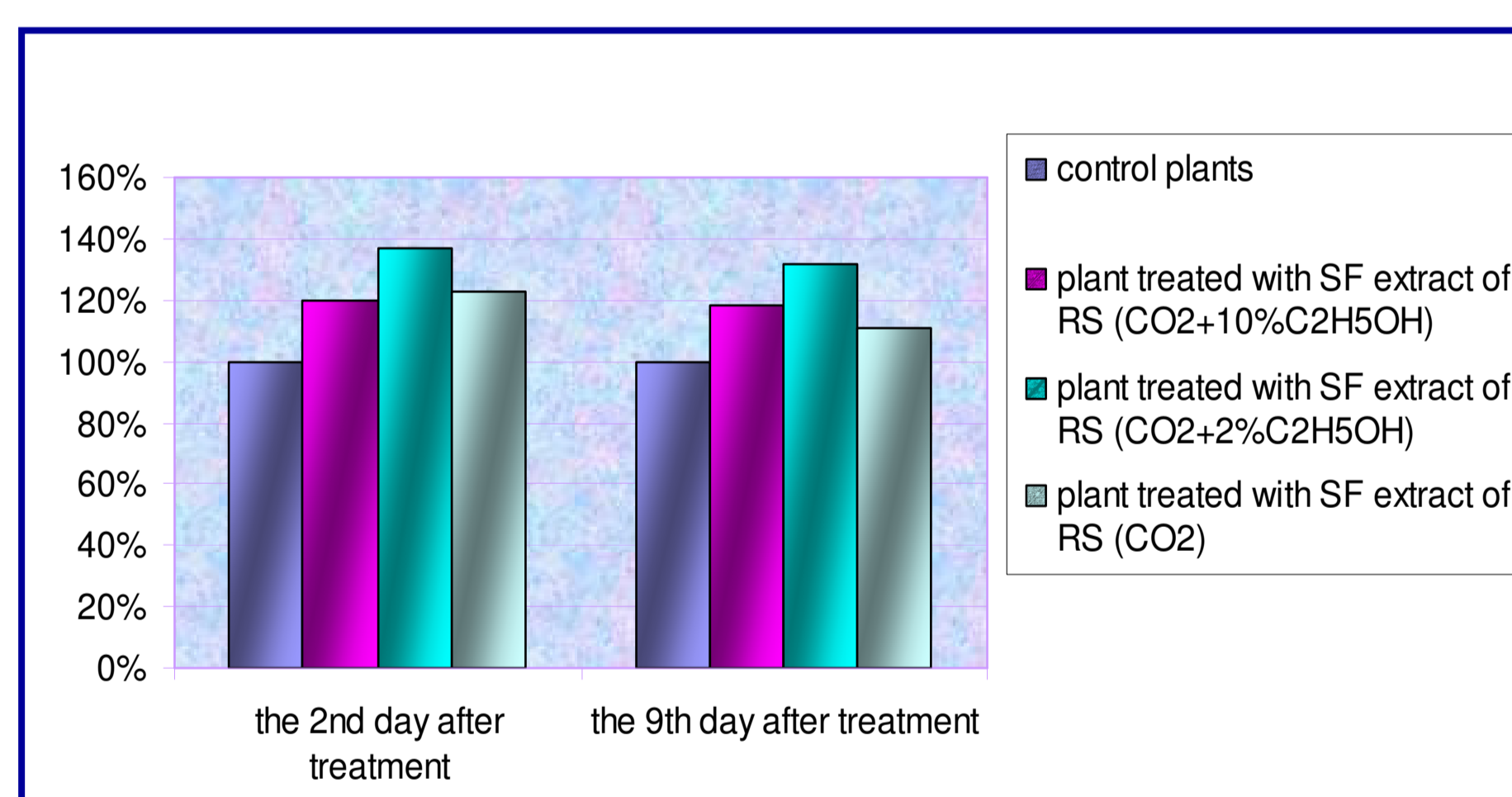
To measure slow fluorescence induction, the leaf was initially adapted to darkness for 5 min and then exposed to wide-band blue light using a slide projector as light source. Fluorescence emission at the wavelength six hundred eighty six nm (the maximum of chlorophyll fluorescence band) was isolated with a monochromator and monitored by a data recording system that included photomultiplier tube and amplifier.

Earlier, it was shown that the ratio  $(F_M - F_T)/F_T$  ( $F_M$  = maximal value,  $F_T$  = stationary level of fluorescence) was correlated with the rate of photosynthetic O<sub>2</sub> evolution per mg chlorophyll.



Slow fluorescence induction (SFI) curves of control plants and plants, treated with supercritical fluid extract (SFE) of *Reynoutria Sachalinensis* (RS)

Spraying of the plants with all preparations resulted in an increase in  $(F_M - F_T)/F_T$  values during approximately 10 days after the treatment indicating the stimulation of photosynthetic activity in bean leaves. Control plants are denoted as hundred percents. Overall, data obtained indicate a positive effect of all types of extracts from *Reynoutria sachalinensis*. Supercritical fluid extract with 10% of ethanol gives an increase in 20% on the 2nd day, and 18% on the 9th day. SF extract with 2% of ethanol shows the best result, 37% on the 2nd day after treatment and 32% on the 9th day after treatment of increasing in comparison with control plants. I need to comment, that we studied different fertilizers and usually they give an increase in 10-15%, maximum 25%. The increase in 37% is maximum we have ever obtained. The supercritical extract obtained only with carbon dioxide as a solvent shows an increase in 23% on the 2nd day after treatment and 11% on the 9th day.



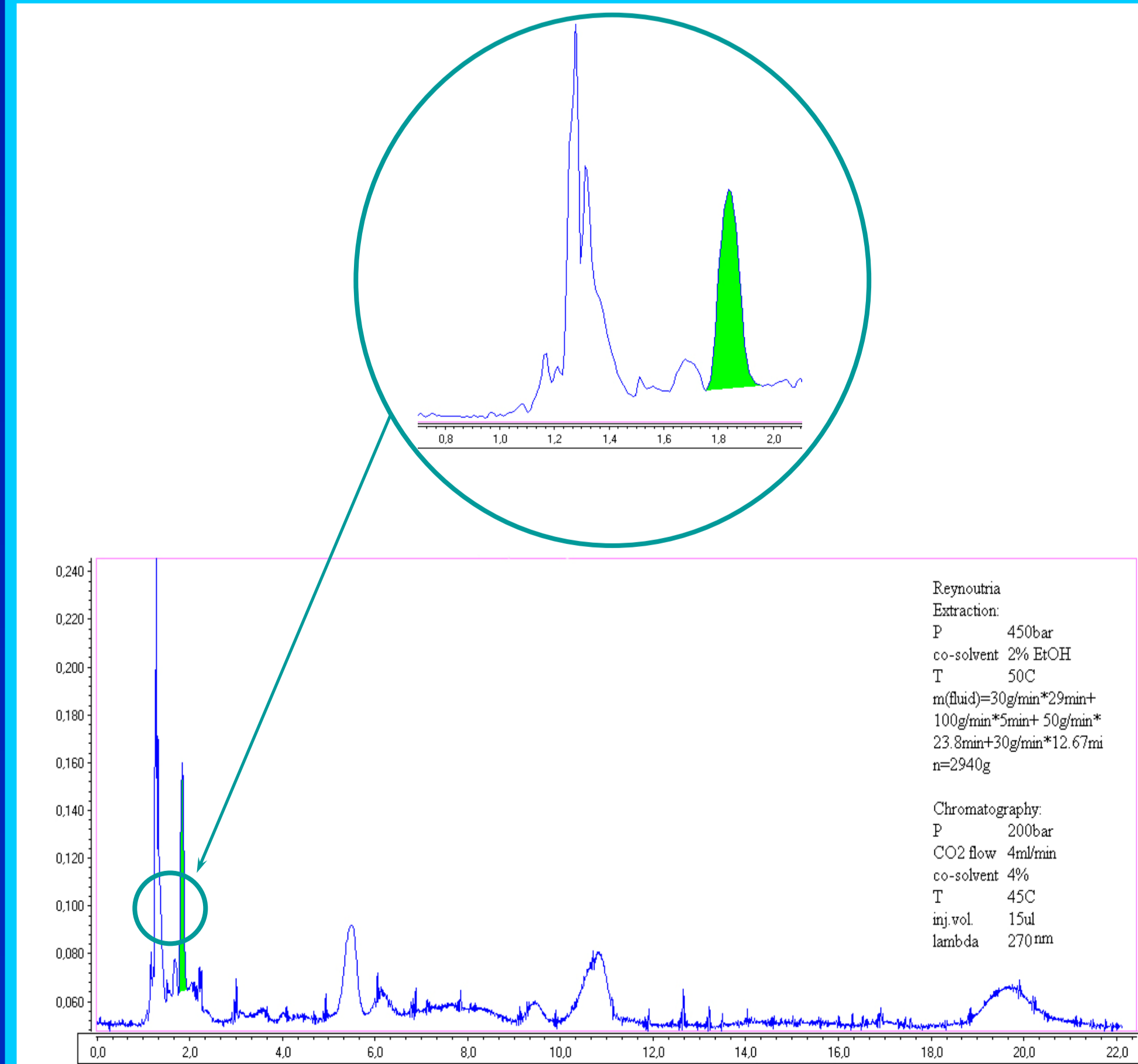
SFI parameters  $((F_M - F_T)/F_T)$  of control and treated plants

Ethanol exerts negative influence on the photosynthetic activities, because it destroys lipid membranes. But we used extracts with low concentration of ethanol. The second type of extract has bigger concentration of active compounds in comparison with the first one, that's why it showed better result. Low concentration of ethanol destroys the chloroplast membranes and allows active compounds to get to stroma. Active compounds of the sprayings induce the resistance and this give an opportunity to membranes to be repaired fast, when they are destroyed incompletely, but slightly, just to let the active compounds to get to stroma.

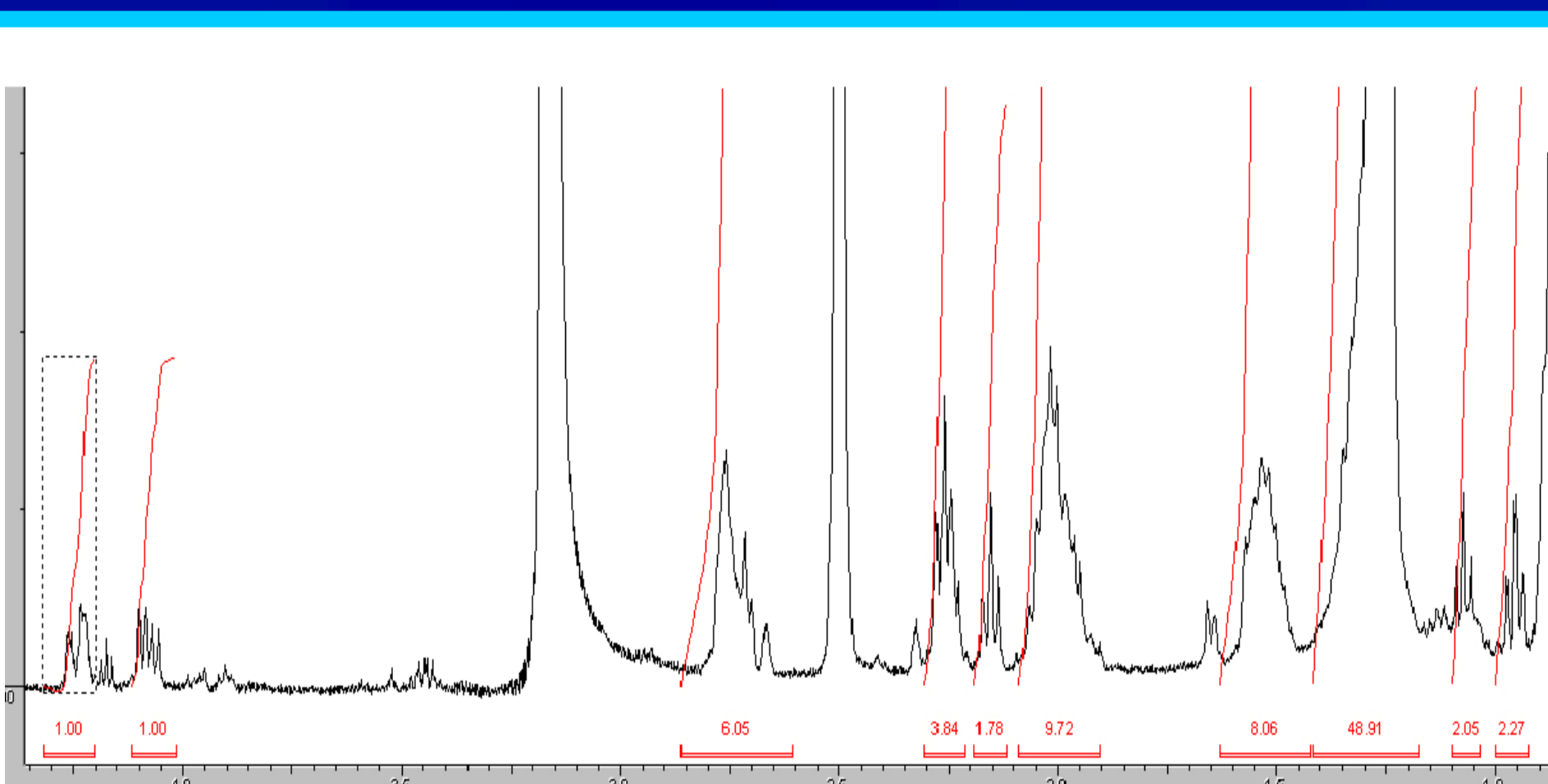


Semi-Prep Investigator by TharSFC

So, we decided to analyze SF extracts of *Reynoutria sachalinensis* by supercritical fluid chromatography (SFC), as SFC is now emerging as the fastest, safest and most environmentally friendly normal phase technique in the chemical industry. Analysis was performed using Thar ASCF chromatograph equipped by a fraction collection system. Ethanol was used as the mobile phase polarity modifier. *Reynoutria sachalinensis* extract appeared to have one major compound which is supposed to be responsible for biological activity of the product. The substance was acquired using Thar semi-preparative silicagel column.



Chromatogram of SF extract of *Reynoutria sachalinensis*



1H NMR spectrum of the peak 1.8 min. Solvent - DMSO-d<sub>6</sub>

According to 1H NMR study of the isolated peak and previous investigations of the matter we can assume that the substance possesses a quinone structure. As NMR analysis of the isolated matter discovered the latter to be a substituted quinone, we could explain the increase of the photosynthetic activity. Quinones supposed to be additional acceptors of electrons in photosynthetic processes. It explains the positive influence of the SF extract on the photosynthetic processes and plants in general. Further research will be held to find out the structure of the compound responsible for the biological activity of *Reynoutria sachalinensis* extracts.

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