СТРЕС РЕАКЦИИ НА ЧЕРЕШОВАТА ПОДЛОЖКА GISELA 5 (*Prunus cerasus* L.× *Prunus canescens* L.) СЛЕД ТРЕТИРАНЕ С ПОЧВЕНИ ХЕРБИЦИДИ: I. ВЛИЯНИЕ ВЪРХУ ФОТОСИНТЕЗАТА И ХЛОРОФИЛНАТА ФЛУОРЕСЦЕНЦИЯ

STRESS RESPONSES OF THE CHERRY DWARF ROOTSTOCK *GISELA* 5 (*Prunus cerasus* L.× *Prunus canescens* L.) AFTER TREATMENT WITH SOIL HERBICIDES: I. EFFECT ON THE PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE

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Резюме

Целта на настоящото проучване беше да се изследват стрес реакциите на вегетативната подложка за череша Гизела 5 след третиране с почвени хербициди в условията на моделен съдов опит. Анализирани са следните варианти: 1. Контрола (нетретирана); 2. Пендиметалин - Стомп 33ЕК-400 ml/da; 3.Изоксафлутол - Мерлин 750 ВГ- 5,0 g/da; 4. Оксифлуорфен - Гоал 2Е-200 ml/da. Проучено е влиянието на тези хербициди върху скоростта на фотосинтезата и параметрите на хлорофилната флуоресценция. Установено е, че третирането с почвените хербициди пендиметалин, изоксафлутол и оксифлуорфен води до значително потискане на фотосинтезата, понижаване на максималната (Fm) и вариабилната (Fv) флуоресценция при всички изследвани растения, което потвърждава наличието на стресови реакции.

Abstract

The aim of the present study was to investigate the stress responses of the vegetative rootstock *Gisela 5* after treatment with soil herbicides under the conditions of a model pot experiment. The following variants were set: 1. control (untreated); 2. *pendimethalin – Stomp 33 EC* – 400 ml/da; 3. *isoxaflutole – Merlin 750 WG* – 5,0 g/da; 4. *oxyfluorfen – Goal 2E* – 200 ml/da. Investigations on the gas exchange rate and the chlorophyll fluorescence after herbicide treatment were carried out. The obtained results showed that the soil herbicides *pendimethalin, isoxaflutole* and *oxyfluorfen* had a negative impact on the gas exchange rate, lowering the maximal (Fm) and variable (Fv) chlorophyll fluorescence, which confirmed the existence of stress responses.

Ключови думи: фитотоксичност, вегетативни подложки.
Key words: phytotoxicity, vegetative rootstocks.

INTRODUCTION

Herbicides are widely used to protect crops against adventitious plants. Nevertheless, a massive introduction of those molecules in the fields can generate negative effects on the environment. Since increasingly more consumers are becoming aware of agricultural practices and their impact on the environment and food quality, pesticide toxicity on non-target crop species is a topic that needs to be investigated. Moreover, herbicide treatments may have secondary adverse effects on non-target plants. Many authors have reported that some herbicides such as 2,4-D, glyphosate, chlorsulfuron or trichloroacetate may cause severe damages to crops by inducing leaf necrosis, an increase in stomatal resistance, inhibition of shoot growth, decrease in germination, accumulation of reactive oxygen species or reduction of net photosynthesis (Bhatti et al., 1997, 1998; Radetski et al., 2000). However, while little is known about the effects of newly synthesized herbicides on crop species, the presence of such molecules in the foliage of non-target crops and in soil has been reported (Jame et al., 1999). Herbicide application in the fruit tree nursery quite often might be risky for the appearance of phytotoxic symptoms in plants (Wazbinska, 1997; Kaufman and Libek, 2000; Rankova, 2004; Rankova, 2006; Rankova et al., 2006). That is why preliminary studies are needed to estimate the effect of different herbicides on the vegetative habits of the rootstocks.

The aim of the present work was to evaluate the stress response of the cherry dwarf rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*) after treatment with the soil herbicides pendimethalin, isoxaflutole and oxyfluorfen.

MATERIAL AND METHODS

Plant Material

The experiment was carried out with *in vitro* propagated and acclimatized to *ex vitro* conditions plants of the vegetative cherry dwarf rootstock Gisela 5 under the conditions of a model pot experiment.

The following variants were set:

1. Control (untreated);
2. Pendimethalin – Stomp 33 EC – 400 ml/da;
3. Isoxaflutole – Merlin 750 WG – 5,0 g/da;
4. Oxyfluorfen – Goal 2E – 200 ml/da.

The rates of the herbicides were recalculated according to the area of the plant pots.

The initial height (mm) of all the plants was measured. The plants were cultivated for 70 days in the greenhouse. Visual observations for the appearance of external symptoms of herbicide phytotoxicity were carried out weekly.

Gas Exchange Measurement

The gas exchange rate of the plants was analyzed by a portable infrared gas analyzer LCA -4 (ADC, UK). The light curves were determined at 4 light intensities (approx. 0, 40, 120 and 250 μmol m^{-2} s^{-1} PPFD) at CO_2-saturation concentration (1500 vpm). Before measuring the dark respiration rate (Rd), the plants were dark adapted for at least 30 min. Using the light curves, the compensation point "Γ" and the maximum net photosynthesis (Pn max) were calculated.

Chlorophyll Fluorescence Analysis

The chlorophyll fluorescence analysis was completed with a chlorophyll fluorometer MINI-PAM (Walz, Germany) on the first fully developed leaves. Minimal fluorescence (Fo) was measured in 45 min dark-adapted leaves using weak modulated light of < 0.15 μmol m^{-2} s^{-1} and maximal fluorescence (Fm) was measured after 0.8 s saturating white light pulse (8000 μmol m^{-2} s^{-1}) in the same leaves. The maximum photochemical efficiency of PS II (maximal quantum yield) was calculated as (Fm"Fo)/Fm=Fv/Fm. In the light adapted leaves, the steady state fluorescence yield F (after a 10-minute illumination with PAR 60 μmol m^{-2} s^{-1}) and the maximal fluorescence (Fm*) after 0.8 s saturating white light pulse (8000 μmol m^{-2} s^{-1}), were determined. Actual quantum yield of PSII in the light-adapted leaves (Y) was calculated by the (Y= (Fm*-F)/Fm*) ratio (Genty et al., 1989). Photochemical (qP) and non-photochemical (qN) quenching parameters were calculated according to Schreiber et al. (1994). The efficiency of the electron transport as a measure of the total photochemical efficiency of PSII (Y) and the rate of the electron transport (ETR) were calculated according to Genty et al. (1989).

Data Analysis

Twelve plants in four replications were set for each variant of herbicide treatment. The results obtained were processed by the dispersion analysis method.

RESULTS

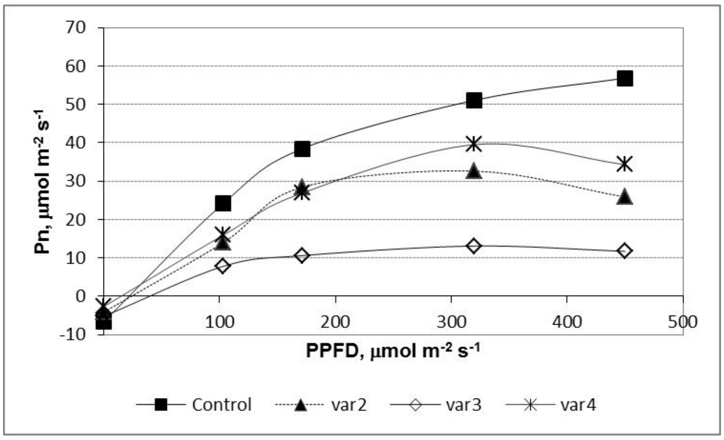
For the control plants, the light saturation of photosynthesis (at CO_2-saturation concentration 1500 vpm) occurred under light intensity of about 300 μmol m^{-2} s^{-1}, the maximum Pn being 56.84 μmolCO_2 m^{-2} s^{-1}, which was higher than for the herbicide treated plants. The gas exchange measurements showed that the treatment with the soil herbicides pendimethalin, oxyfluorfen and isoxaflutole led to a significant reduction in CO_2 assimilation. The maximal net photosynthetic rate at saturated CO_2 and saturated light of plants treated with pendimethalin and oxyfluorfen was about 30% lower than the control, and 59% lower in isoxaflutole treated plants (Table 1).

A light intensity of about 250 μmol m^{-2} s^{-1} was saturating for the plants of the herbicide treated variants, while in the control the saturation was reached about 300 μmol m^{-2} s^{-1} PPFD (Fig. 1).

A significant difference was also observed in the light compensation point, the value for the plants treated with isoxaflutole being over 36 μmol m^{-2} s^{-1}, i.e. two times higher than for the control (Table 2). The depressing effect of the studied herbicides on the photosynthesis of cherry plants was confirmed by the parameters of chlorophyll fluorescence. The reduced maximal fluorescence (Fm) in all the treated plants may indicate the increase in a non-photochemical quenching process at or close to the reaction center (Baker and Horton, 1987), (Table 3). These processes were especially pronounced in plants, treated with isoxaflutole. In this variant, the Fv/Fm ratio, which characterizes the maximum quantum yield of the primary photochemical reactions in dark adapted leaves, was significantly changed.

The efficiency of the electron transport as a measure of the total photochemical efficiency of PSII (Y) decreased considerably in the leaves of the plants treated with isoxaflutole and pendimethalin, while in the plants treated with oxyfluorfen it was less affected (Table 3). Photochemical quenching (qP) presented a similar behavior to Y (Table 3.).

In the previous part of our experiment it was observed that although no external symptoms of phytotoxicity or growth suppression were recorded, a lower value of leaf pigments content was established after treatment with isoxaflutole. It was proven that the reduction



***Фиг. 1.** Влияние на почвените хербициди пендиметалин, изоксафлутол и оксифлуорфен върху скоростта на газообмена на растения от Гизела 5 при насищаща CO_2 -концентрация (1500 vpm)*

***Fig. 1**. Effect of the soil herbicides pendimethalin, isoxaflutole and oxyfluorfen on the gas exchange rate of Gisela 5 plants at saturated CO_2-concentration (1500 vpm)*

Control (■); pendimethalin (▲); isoxaflutol (✳); oxyfluorfen (◇)

Таблица 1. Стойности на функционалните фотосинтетични параметри, изчислени от светлинните криви при насищаща CO_2 -концентрация (1500 vpm) на контролата и третираните с хербициди растения от Гизела 5

Table 1. Values of the functional photosynthetic parameters calculated from the curves of net photosynthetic rate versus photon flux density (Pn=f (PAR)) at saturated CO_2-concentration (1500 vpm) of control and herbicide treated Gisela 5 plants

Варианти/ Variant	Pn max	Светлинна комп. точка/ Light comp. point, Г	Тъмнинно дишане/ Rd, dark respiration
Control	56.84	18.4	-6.6
Var 2	35.8	14.9	-3.5
Var 3	17.63	36	-5.54
Var 4	38.47	0.4	-1.24

Таблица 2. Параметри на хлорофилната флуоресценция на тъмнинно адаптирани листа на контролата и третираните с хербициди растения от Гизела 5

Table 2. Parameters of chlorophyll fluorescence in dark adapted leaves of control and herbicide treated Gisela 5 plants. Different letters within each column indicates significant difference ($P<0.05$) by DMRT (Duncan's Multiple Range Test)

Варианти Variants	F_0	Fm	Fv	Fv/Fm
Control	837±25a	3109±91a	2272±83a	0.73 ±0.035a
Var 2	688±21c	2504±87b	1816±97b	0.725±0.033a
Var 3	1223±27d	2327±92c	1104±99c	0.474±0.081b
Var 4	736±22b	2526±96b	1790±98b	0.708±0.032a

Таблица 3. Параметри на хлорофилната флуоресценция на светлинно адаптирани листа на контролата и третираните с хербициди растения от Гизела 5

Table 3. Parameters of chlorophyll fluorescence in light adapted leaves of control and herbicide treated Gisela 5 plants. Different letters within each column indicates significant difference ($P<0.05$) by DMRT (Duncan's Multiple Range Test)

Варианти Variants	Yield (Y)	ETR	qP	qN	NPQ
Control	0.638±0.021a	4.2	0.972	0.295	0.275
Var 2	0.501±0.023b	2.9	0.722	0.142	0.114
Var 3	0.302±0.029c	2.1	0.675	0.103	0.051
Var 4	0.620±0.027a	3.8	0.905	0.106	0.081

of the pigment concentration is an indicator of stress (Hendry and Grime, 1993).

The obtained results of the studied physiological parameters showed that the soil herbicides pendimethalin, isoxaflutole and oxyfluorfen had a negative impact on photosynthesis, confirmed by a reduction in the gas exchange rate and chlorophyll fluorescence. This result is in agreement with those observed in *Vitis* after treatment with herbicide flumioxazin (Saladin et al., 2003). Those results provide new insights into the physiological responses of nontarget crops to herbicide treatments.

CONCLUSIONS

Treatment with the soil herbicides pendimethalin, oxyfluorfen and isoxaflutole leads to a significant inhibition of photosynthesis and the yield of chlorophyll fluorescence, mainly in plants treated with isoxaflutole. Those results confirm the existence of plant stress responses after treatment with the soil herbicides although no external symptoms of phytotoxicity or growth suppression is recorded.

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