СТРЕС РЕАКЦИИ НА ЧЕРЕШОВАТА ПОДЛОЖКА GISELA 5 (*Prunus cerasus* L.× *Prunus canescens* L.) СЛЕД ТРЕТИРАНЕ С ПОЧВЕНИ ХЕРБИЦИДИ: II. ВЛИЯНИЕ ВЪРХУ РАСТЕЖА И СЪДЪРЖАНИЕТО НА ФОТОСИНТЕТИЧНИ ПИГМЕНТИ

STRESS RESPONSES OF THE CHERRY DWARF ROOTSTOCK *GISELA* 5 (*Prunus cerasus* L.× *Prunus canescens* L.) AFTER TREATMENT WITH SOIL HERBICIDES: II. EFFECT ON THE GROWTH AND PHOTOSYNTHETIC PIGMENTS

Заря Ранкова[1]*, Лиляна Начева[1], Петя Герчева[1], Златко Златев[2]
Zarya Rankova[1*], Lilyana Nacheva[1], Petya Gercheva[1], Zlatko Zlatev[2]

[1] Институт по овощарство – Пловдив
[2] Аграрен университет – Пловдив
[1] Fruit-Growing Institute – Plovdiv
[2] Agricultural University – Plovdiv

***E-mail: rankova_zarya@abv.bg**

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. Дозата на хербицидите беше преизчислена спрямо площта на култивационния съд. Проведени са ежеседмични визуални наблюдения за поява на външни симптоми на фитотоксичност от хербицидите. На 70-тия ден се отчетоха биометричните показатели прираст, средна маса на растение, брой възли, среден брой листа на растение и средна маса на листата. Извършен беше анализ за съдържанието на листни пигменти (хлорофил и каротиноиди). Установено е, че третирането с почвения хербицид изоксафлутол предизвиква хлороза, както и понижено съдържание на листни пигменти при черешовата подложка Гизела 5.

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. The herbicide rates were recalculated according to the area of the plant pots. Visual observations for the appearance of external symptoms of herbicide phytotoxicity were carried out weekly. The biometric characteristics – growth increment, mean weight of a plant, mean number of leaves and nodes and chlorophyll content, were reported on the 70th day. The obtained results showed that the soil herbicide *isoxaflutole* caused white chlorosis and lower content of leaf pigments in the cherry rootstock *Gisela 5*.

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 the 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 was 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 et al., 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* L.× *Prunus canescens* L.) 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. Isoxaflutol – 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 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. At the end of the period (on the 70th day) the following biometric indices were reported – growth increment (cm), mean plant weight (g), mean weight of the leaves (g), number of newly developed nodes and mean number of leaves per plant.

Chlorophyll Content

The plastid pigments (chlorophyll and carotenoids) content was determined spectrophotometrically in 80% acetone extract. The amount of the leaf pigments was calculated according to the Lichtenthaler and Wellburn formula (1983).

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

External symptoms of phytotoxicity – chlorosis, necrosis or growth suppression were not observed in the plants treated with pendimethalin (Variant 2). The plants in that variant did not differ in external characteristics from those of the untreated control.

On the 7th day, slight white chlorosis was observed in the lower leaves of the plants treated with isoxaflutole (Variant 3). Those symptoms did not appear in the apical leaves. 30 days after treatment those symptoms of phytotoxicity were not observed. The new growth was vigorous, showing that the plants had overcome the stress of the herbicide treatment.

On the 10th–15th day slight necrosis appeared in the leaves of the plants in Variant 4 (oxyfluorfen – Goal 2E).

White chlorosis is a typical symptom of phytotoxicity in plants susceptible to isoxaflutole. In previous studies, very strong white chlorosis was detected, leading to whitening of the entire plants of yellow plum after treatment with Merlin 750 WG - 5,0 g/da under *in vitro* conditions (Rankova et al., 2004). The phytotoxic effect of isoxaflutole was established in the cotyledons and leaves of embryocultures of yellow plum and it was due to the mechanism of its herbicide effect. It is absorbed by the young germs and roots and attacks the meristem tissues. The active substance can attack the enzymes responsible for the synthesis of chlorophyll and carotenoids (Tonev, 2000).

On the 70th day, growth depression and withering of the vegetative tip were not observed in the plants of the treated variants.

The vegetative tip in all the plants was fresh and vigorously growing. That gave the grounds to conclude that the soil herbicides included in the present study, did not cause external symptoms of growth suppression in the vegetative rootstock Gisela 5 (Fig. 1).

The results of the biometric analysis are presented in Fig. 2 and 3. The plants of the variants treated with herbicides, had growth increment values similar or higher compared to the control. The highest increment length was reported in the plants of Variant 4 – oxyfluorfen – Goal 2E - 200 ml/da, the differences to the control being statistically highly significant. Similar results were obtained about the effect of the studied soil herbicides on plant weight (Fig.3).

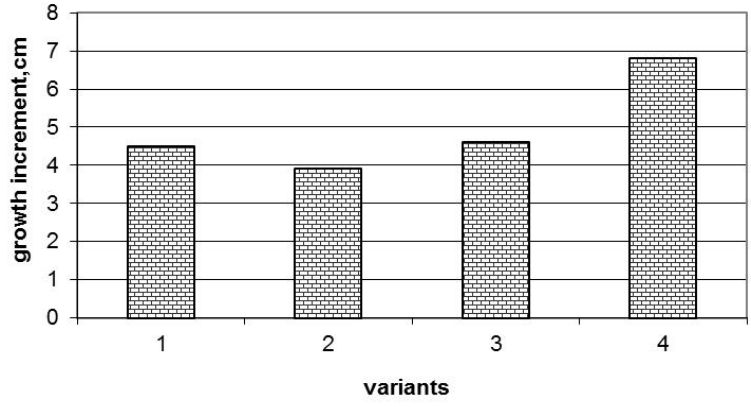
The plants in Variants 2 and 3 showed a tendency to a lower value of the above-ground plant weight compared to the control. The differences were statistically insignificant. That allows drawing the conclusion that the soil herbicides included in the study, do not suppress the growth of the vegetative rootstock Gisela 5.

A significant effect of the soil herbicides on the number of internodes and the mean number of leaves per plant was not reported, confirming the lack of a depressing effect on that characteristic, exerted by the active substances (Fig. 4).

Data about the content of leaf pigments are presented in Table 1. Lower values of leaf pigment contents were reported in the plants of Variant 3 – isoxaflutole – Merlin 750 WG – 5,0 g/da. It was probably due to the

Фиг. 1. *Влияние на някои почвени хербициди върху растежа на подложката Gisela 5. 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*

Fig. 1. *Effect of some soil herbicides on growth of Gisela 5 plants. Variants: 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*



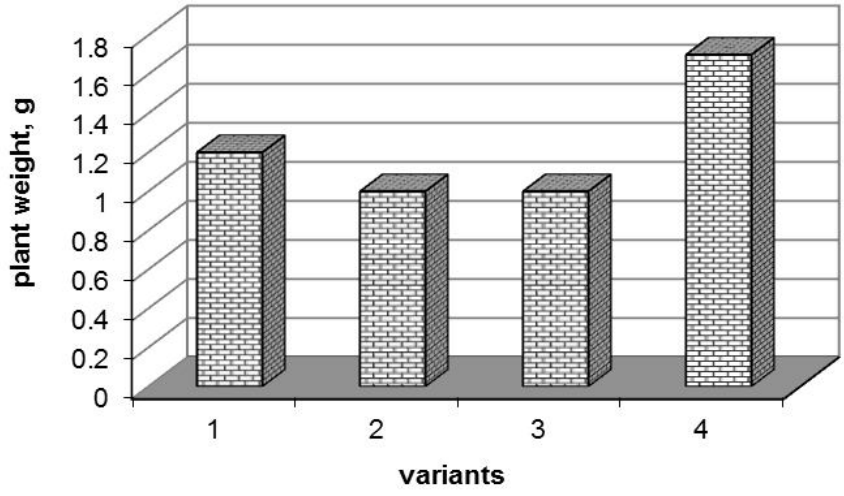
Фиг. 2. *Влияние на някои почвени хербициди върху прираста на подложката Gisela 5. Варианти: 1. Контрола (нетретирана); 2. Пендиметалин - Стомп 33ЕК - 400 ml/da; 3.Изоксафлутол - Мерлин 750 ВГ - 5,0 g/da; 4. Оксифлуорфен - Гоал 2Е - 200 ml/da*

Fig. 2. *Effect of some soil herbicides on growth increment (cm) of Gisela 5 plants. Variants: 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 LSD 5% – 0,5; 1% – 0,7; 0,1% – 1,0*

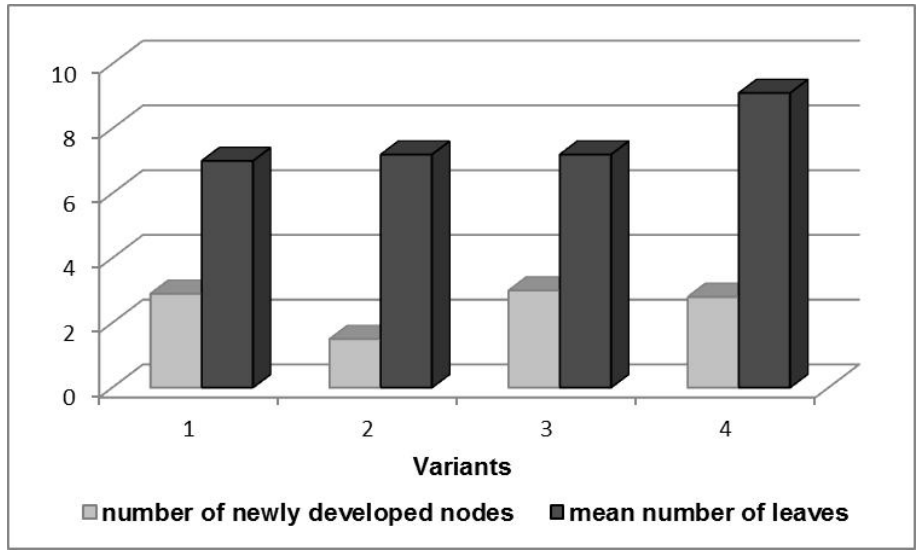
Таблица 1. Влияние на почвените хербициди върху съдържанието на листни пигменти в листата при вегетативната подложка Gisela 5

Table 1. Effect of the soil herbicides on the content of plastid pigments (chlorophyll and carotenoids) in the leaves of Gisela 5 plants

Варианти Variants	Абс. суха маса, % Absolute dry matter, %	Съдържание на хлорофил Chlorophyll content (mg/g_{DW})			Съдържание на каротеноиди Carotenoids content (mg/ g_{DW})
		а	в	а+в	
1	25,55	6,16	3,11	9,40	0,48
2	25,49	6,75	2,93	9,69	0,52
3	22,92	5,20	2,59	7,79	0,45
4	24,10	7,56	3,29	10,85	0,60



Фиг. 3. *Влияние на някои почвени хербициди върху средната маса на едно растение при вегетативната подложка Gisela 5.*
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
Fig. 3. *Effect of some soil herbicides on the mean plant weight (g) of Gisela 5 plants.*
Variants: 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
LSD 5% - 0,7; 1% - 1,0; 0,1% - 1,4



Фиг. 4. *Влияние на някои почвени хербициди върху броя на междувъзлията и средния брой листа на едно растение при вегетативната подложка Gisela 5*
Fig. 4. *Effect of some soil herbicides on the number of newly developed nodes and on the mean number of leaves per plant.*
Variants: *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

number of newly developed nodes - LSD 5% - 1,6; 1% - 2,2; 0,1% - 3,3
mean number of leaves per plant - LSD 5% - 2,3; 1% - 3,3; 0,1% - 4,6

mechanism of action of the active substance and its capacity to attack the enzymes responsible for the synthesis of chlorophyll and carotenoids.

In Variant 2 the leaf pigment values were close to those in the control. The highest content of chlorophyll a, в, (а+в) and carotenoids was reported for the plants in Variant 4 – oxyfluorfen – Goal 2E – 200 ml/da. Most probably it was due to the contact mode of action of oxyfluorfen and the lack of an effect on the formation of leaf pigments.

CONCLUSIONS

1. Treatment with soil herbicides pendimethalin and oxyfluorfen did not cause external symptoms of phytotoxicity. Application of isoxaflutole (Merlin 750 WG – 5,0 g/da) caused the appearance of external symptoms of phytotoxicity (white chlorosis), which was overcome about 30 days after treatment;
2. Vegetative growth depression was not established in the cherry rootstock Gisela 5 after treatment with the soil herbicides pendimethalin, isoxaflutole and oxyfluorfen at the applied rates;
3. Lower values of leaf pigments content was established after treatment with isoxaflutole.

REFERENCES

Bhatti, M., K. Al-Khatib, R. Parker, 1997. Wine grape (*Vitis vinifera* L.) response to fall exposure of simulated drift from selected herbicides. – Weed Technol, 11:532–536.

Bhatti, M., A. Felsot, R. Parker, G. Mink, 1998. Leaf photosynthesis, stomatal resistance, and growth of wine grapes (*Vitis vinifera* L.) after exposure to simulated chlorsulfuron drift. – J Environ Sci Health B, 33: 67–81.

Jame, Y., A. Cessna, V. Biederbeck, R. Grover, A. Smith, H. Korven, 1999. Herbicide residues and yield effects from repeated flood-irrigations of alfalfa with water containing monouron or simazine. – Can J Plant Sci, 79: 639–645.

Kaufman, E., A. Libek, 2000. Damages to cherry plum seedlings *(Prunus cerasifera var. Daviricata Bailey)* caused by herbicides. – In: Proceedings of the International Conference on Fruit Production and Fruit Breeding, Tartu, Estonia, 12-13 September, 132-137.

Lichtenthaler, H., A. Wellburn, 1983. Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. Trans, 603: 591-592.

Radetski, C., S. Cotelle, J. Ferard, 2000. Classical and biochemical endpoints in the evaluation of phytotoxic effects caused by the herbicide trichloroacetate. – Environ Exp Bot, 44: 221–229.

Rankova, Z., 2006. Effect of some soil herbicides on the vegetative habits of mahaleb cherry *(Prunus machaleb L.)* seedling rootstocks. – Bulg. J. Agric. Sci., 12: 429-433.

Rankova, Z., P. Gercheva, K. Ivanova, 2004. Screening of soil herbicides under in vitro conditions. – Acta Horticulturae Serbica, vol. IX, 17: 11-17.

Rankova, Z., L. Nacheva, K.Zapryanova, P.Gercheva, V. Bozkova, 2006. Effect of soil herbicides napropamid and pendimethalin on rooting and growth of the vegetative plum rootstock Pr. domestica Wangenheims under in vitro conditions. – Journal of mountain agriculture on the Balkans, 9(3): 349-359.

Tonev, T., 2000. Handbook of integrated weed control and culture of farming, Book 2, Higher Institute of Agriculture, Plovdiv, p. 275, 126-127.

Wazbinska, J., 1997. Technological improvement of generative cherry plum rootstocks, one-year Wegierka Lowicka plum trees and apple seedlings. – Acta. Academiae Agriculturae ac Technicae, Olstenensis Agricultura, 64: 107.

ACKNOWLEDGEMENTS

This research is a part of the projects № RNF 01/0106 and № DO 02-88/2008, supported by National Science Fund, Ministry of Education and Science, Bulgaria.

Статията е приета на 20.12.2011 г.
Рецензент – доц. д-р Невена Стоева
E-mail: stoevanevena@abv.bg