Bulgarian Journal of Agricultural Science, 21 (No 6) 2015, 1194-1197
Agricultural Academy

BEHAVIOUR OF THE SOUR-SWEET CHERRY HYBRID 20-192 AFTER TREATMENT WITH SOIL HERBICIDES UNDER *IN VITRO* CONDITIONS

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Abstract

RANKOVA, Z., L. NACHEVA, A. ZHIVONDOV and P. GERCHEVA, 2015. Behaviour of the sour-sweet cherry hybrid 20-192 after treatment with soil herbicides under *in vitro* conditions. *Bulg. J. Agric. Sci.*, 21: 1194–1197

The experiment was carried out at the Laboratory of Biotechnology within the Fruit-Growing Institute – Plovdiv with *in vitro* propagated and rooted plants of the newly bred rootstock for sweet and sour cherry 20-192. The interspecific hybrid No. 20-192 (*Prunus cerasus* L.× *Prunus avium* L.) was introduced into culture and propagated *in vitro* in MS nutrient medium (Murashige and Skoog, 1962) supplemented with BAP и IBA. Root-formation was established after about 20 days. Treatment with herbicides was carried out under *in vitro* conditions 35 days after culturing in the rooting medium when the rootlets reached a length of about 2 cm. The effect of the soil herbicides napropamid (Devrinol 4F), pendimethalin (Stomp 33 EC) and oxyfluorfen + metolachlor (Metofen), each of them applied at three different doses, was studied with the aim of developing a system of rapid preliminary screening. The results obtained showed that after treatment with the soil herbicides napropamid and pendimethalin under *in vitro* conditions, visual symptoms of phytotoxicity and growth suppression were not observed in the plants of the vegetative rootstock 20-192. Strong phytotoxicity expressed in necrosis and dying of the plants was observed after treatment with high doses of Metofen. The studied herbicides did not exert a depressing effect on stem growth at the doses used. After treatment with high doses of napropamid, plant growth suppression was established, expressed in a lower mean weight of a plant.

Key words: cherry rootstocks, soil herbicides, *in vitro*, phytotoxicity, growth habits

Abbreviations: BAP - 6-benzylaminopurine; IBA - indole-3-butyric acid

Introduction

Weed control in the fruit tree nursery is the major agro technical problem, to a large extent determining the success in the production of top quality planting material of a good health state. It is known that the separate fruit species used as rootstocks show different level of susceptibility after treatment with herbicides. Strong phytotoxicity after applying herbicides, expressed in growth suppression and dying of the plants was reported in some experiments, as well as no negative effect on plant growth and development was observed in other experiments, in which good quality rootstocks were obtained, suitable to be grafted in the year of planting (Wazbinska, 1997; Abdul et al., 1998; Kaufman and Libek, 2000a; Kaufman and Libek, 2000b; Rankova, 2002, 2006, 2007, 2008; Popov and Rankova, 2009; Rankova, 2010, 2011;Rankova et al., 2012; Rankova and Tityanov, 2013; Rankova and Zhivondov, 2013). In that aspect, developing an *in vitro* test system of rapid preliminary screening for the evaluation of the possible effect of the soil herbicides on the growth of rootstocks is a reliable method, which enables getting preliminary information about the plant habit and the expected stress response (Gercheva et al., 2002; Rankova et al., 2006; Rankova et al., 2009; Nacheva et al., 2012; Rankova et al., 2012).

The aim of the present experiment was to study the behaviour of the interspecific hybrid 20-192 after treatment with soil herbicides under *in vitro* conditions, tested as a rootstock of poor growth vigour for sweet and sour cherry cultivars.

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Materials and Methods

Studies were carried out with *in vitro* propagated and rooted plants of the interspecific hybrid No. 20-192, at the Laboratory of Biotechnologies within the Fruit-Growing Institute – Plovdiv.

Hybrid No. 20-192 was established at the Fruit-Growing Institute – Plovdiv in result of the implemented programme for breeding rootstocks of poor growth vigour for sweet and sour cherry cultivars. It was obtained from crossing the low-vigour sour cherry cultivar 'Polevka' (*Prunus cerasus* L.) with pollen of the sweet cherry cultivar 'Compact Van' (*Prunus avium* L.). The source plant is a twenty-four year old tree with good vitality, grown under non-irrigation conditions and without plant protection treatments, those facts showing its drought resistance and resistance to diseases and pests. It is 140 cm in height, with a semi-drooping crown habit. It is easily propagated under *in vitro* conditions. In the nursery, the rootstocks reach the optimal thickness at the place of grafting in the first half of September. At present, the hybrid No. 20-192 has been studied in the nursery as a perspective one, characterized by its poor growth vigour, suitable for sweet and sour cherry cultivars (Zhivondov, 2012; Plumcots).

Hybrid No. 20-192 was introduced into culture and *in vitro* propagated in MS (Murashige and Skoog, 1962) nutrient medium supplemented with BAP и IBA.

While conducting the experiment, single microplants of the hybrid 20-192 were cultured for elongation in MS nutrient medium without growth regulators for 15 days. The plantlets about 2 cm in height, were rooted in MS nutrient medium with a half-reduced concentration of macroelements, 0.2 mg/l IBA, 20 g/l sucrose, 6.5 $g.l^{-1}$ agar, pH 5.6. Root formation was observed after about 20 days. Treatment with herbicides was applied under *in vitro* conditions 35 days after putting the plantlets in the medium for rooting, when the rootlets were about 2 cm in length.

The effect of the soil herbicides napropamid (Devrinol 4F), pendimethalin (Stomp 33 EC) and oxyfluorfen + metolachlor (Metofen) was studied with the aim of developing a system of rapid preliminary screening. The following variants were set: 1. Control (untreated); 2. Napropamid – Devrinol 4F – 300 ml/da; 3. Napropamid – Devrinol 4F – 400 ml/da; 4. Napropamid – Devrinol 4F – 500 ml/da; 5. Pendimethalin – Stomp 33 EC – 300 ml/da; 6. Pendimethalin – Stomp 33 EC – 400 ml/da; 7. Pendimethalin – Stomp 33 EC – 500 ml/da; 8. Oxyfluorfen + metolachlor – Metofen – 160 ml/da; 9. Oxyfluorfen + metolachlor – Metofen – 200 ml/da; 10. Oxyfluorfen + metolachlor – Metofen – 240 ml/da. The herbicide doses were calculated according to the area of the cultivation vessels. The herbicide solution was dropped onto the nutrient medium surface. All the *in vitro* plants were cultivated in a growth chamber at a temperature of 22 ± 2^0C and a photoperiod of 16/8 h (40 μmol m^{-2} s^{-1} PAR).

Plantlets were cultivated for 30 days after treatment with the herbicides. During that time observations were carried out to establish eventual external symptoms of phytotoxicity (chlorosis, necrosis, obvious disturbances in plant development). The biometric characteristics stem height (h-cm) and mean weight of a plant (g) were reported on the 30th day.

Results and Discussion

On the seventh day after treatment with the herbicides, visual symptoms of phytotoxicity were not observed. The plants treated with the herbicides did not differ from the control. In all the variants, the vegetative tips of the plants were fresh and actively growing. On the 14th day, the plants treated with the higher dose of Metofen (Var. 10) developed necrosis on the leaves. Phytotoxicity was not established in the other variants. On the 21st day necrosis appeared in the three variants treated with Metofen, most obviously expressed in the variant with the highest dose (Var. 10) (Figure 1). Necrosis spread to the entire plant and plants started drying out (Figure 2).

The results of the biometric analysis showed that the studied herbicides at the applied doses did not have a depressing effect on the stem growth (Figure 3). The reported values of those characteristics were close to the control. In the three variants treated with Metofen, in which external symptoms of phytotoxicity and drying out of the plants were observed (Var. 8, 9 and 10), the height was close to or higher than the control. A tendency to a decrease of the stem height with the increase of the Metofen dose was established. Analogous data were reported about the effect of Metofen on the mean weight

Fig. 1. Necrosis in *in vitro* plantlets of hybrid 20-192 treated with Metofen (Var. 8, 9 and 10)

of a plant (Figure 4). The herbicides included in the study did not cause a reduction of the mean plant weight. Lower values of the characteristic were established in the plants treated with the high doses of napropamid (Var. 4), which signals growth suppression. In the rest of the variants the values of the mean plant weight were close to the control.

Fig. 2. Dried out plants of hybrid 20-192 after treatment with Metofen (Var. 10), (on the 30th day)

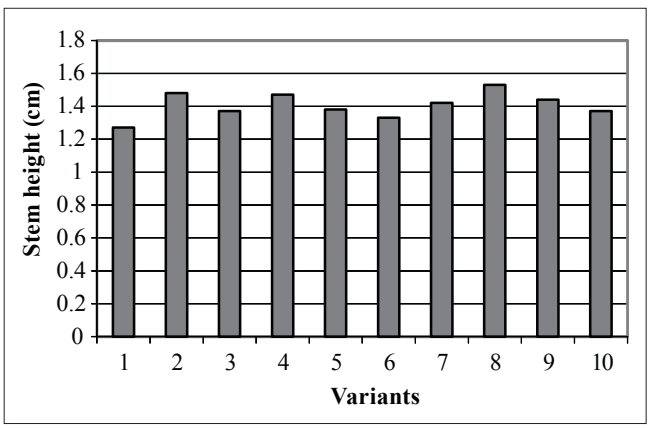

Fig. 3. Effect of soil herbicides on the plant height of hybrid 20-192 (h-cm)

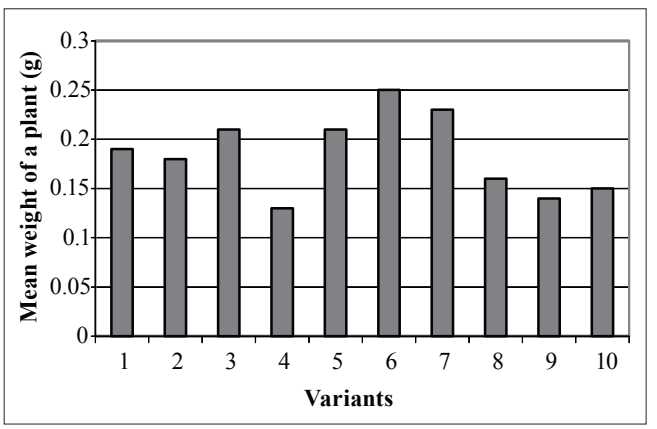

Fig. 4. Effect of soil herbicides on the mean weight of a plant of hybrid 20-192 (g)

The obtained results about the lack of a suppressing effect of pendimethalin and napropamid under *in vitro* conditions were analogous to the behaviour of hybrid 20-192, studied after treatment with soil herbicides under the conditions of a pot experiment (Rankova, unpublished data). Phytotoxicity caused by Metofen was not observed under those conditions. The severe phytotoxicity caused by Metofen under *in vitro* conditions could be explained by the activity of oxyfluorfen, the active substance contained in the commercial product Metofen, because of its contact toxic effect.

Conclusions

- Visual symptoms of phytotoxicity and growth suppression of hybrid 20-192 plants were not observed after treatment with the soil herbicides napropamid and pendimethalin under *in vitro* conditions.
- Severe phytotoxicity expressed as necrosis and dying of the plants was reported after treatment with a high dose of Metofen.
- The studied herbicides at the doses applied, did not show a suppressing effect on stem growth. Plant growth suppression was established after treatment with a high dose of napropamid, resulting in a reduced mean weight of a plant.

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Received September, 14, 2015; accepted for printing October, 5, 2015