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IN VITRO МОДЕЛНА СИСТЕМА ЗА ОЦЕНКА НА СТРЕСОВИЯ ОТГОВОР НА ОВОЩНИ РАСТЕНИЯ КЪМ ТРЕТИРАНЕ С ПОЧВЕНИ ХЕРБИЦИДИ
IN VITRO MODEL SYSTEM FOR EVALUATION OF FRUIT PLANTS STRESS RESPONSES TO SOIL HERBICIDE TREATMENT

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Резюме

Приложението на хербициди в овощните разсадници като елемент от добрата агротехника често крие риск от проявяване на фитотоксични симптоми при растенията. Затова са необходими предварителни изследвания за въздействието на различните хербициди върху вегетативните прояви на подложките. Целта на настоящото изследване бе да се проследи влиянието на почвените хербициди тербацил, пендиметалин и напропамид върху изолирани ембриони от *Prunus cerasifera* и някои микроразмножени клонови подложки за овощни видове - GF677, ММ106 и *Prunus domestica* "Wangenheims" при *in vitro* условия. След тертиране с пендиметалин е установено инхибиране на кореновите меристеми и покафеняване на котиледоните на ембриони от *Prunus cerasifera* с дължина на ембрионалния корен, по-малка от 5 mm. Почвените хербициди тербацил, пендиметалин и напропамид причиняват фитотоксичност, изразяваща се в потискане на растежа на корените на вкоренени растения от GF677 при *in vitro* условия. Визуални симптоми на фитотоксичност по листата и стъблата не са наблюдавани. Пендиметалин и напропамид депресират растежа на стъблата и корените на микрорастения от ябълковата подложка ММ106.

След третиране с тербацил на *in vitro* вкоренени растения от подложката "Wangenheims" не са отчетени симптоми на фитотоксичност – хлороза, некроза или забавяне на растежа. Третирането с пендиметалин и напропамид потиска формирането на корени и растежа на невкоренени микрорастения. Тези два хербицида блокират вкореняването на подложката *Prunus domestica* "Wangenheims" при *in vitro* условия.

Abstract

Herbicide application in the fruit tree nursery as an element of good agrotechnical practice quite often might be risky for the appearance of phytotoxic symptoms in plants. 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 research was to study the effect of the soil herbicides *Terbacil, Pendimethalin* and *Napropamide* on isolated *Prunus cerasifera* embryos and some micropropagated rootstocks for fruit species - GF677, MM106 and *Prunus domestica* "Wangenheims" under *in vitro* conditions. Inhibition of root meristem growth and browning of cotyledons were established after the treatment with pendimethalin of *Prunus* embryos with embryonic roots shorter than 5mm. The soil herbicides terbacil, pendimethalin and napropamid caused phytotoxicity expressed in suppression of the root growth of rooted plants of GF677 under *in vitro* conditions. Visual symptoms of phytotoxicity in the leaves and stems were not established. Pendimethalin and napropamid depressed the stem and root growth of the apple rootstock MM106 plantlets. After treatment with terbacil of *in vitro* rooted "Wangenheims" plants no external symptoms of phytotoxicity – chlorosis, necrosis and depressing effect were observed. After treatment with pendimethalin and napropamide, a depressing effect on the root formation and growth of microplants without roots were established. The applied herbicides blocked rooting of *Prunus domestica* "Wangenheims" under *in vitro* conditions.

Ключови думи: почвени хербициди, фитотоксичност, вегетативни подложки, in vitro, ембриони.
Key words: soil herbicides, phytotoxicity, vegetative rootstock, in vitro, embryos.

INTRODUCTION

Herbicide application in the fruit tree nursery as an element of good agrotechnical practice quite often might be risky for the appearance of phytotoxic symptoms in plants (Wazbinska, 1997; Kaufman and Libek, 2000; Rankova, 2004; Rankova, 2006). That is why preliminary studies are needed to estimate the effect of different herbicides on the vegetative habits of the rootstocks.

The *in vitro* plants are useful experimental model system for evaluating the effect of different environmental factors. They are grown under controlled conditions, they are uniform, they can be reproduced quickly and easily in great quantities and they enable precise experiments for evaluation of different parameters – growth, biomass accumulation, biochemical and biophysical indices about the physiological status of the plants. Although observations on growth characteristics and physiological status of the treated plants have been carried out under field conditions, the use of *in vitro* plants gives a new opinion about the mechanism of action of the bioactive substances (Rankova et al., 2004). A similar model system was used in studying the physiological effect of heavy metals on agricultural crops (Costa and Spitz, 1977; Sanita di Toppi et al., 1998).The results obtained in *in vitro* screening were confirmed by *in vivo* observations (Saladin et al., 2003).

The aim of the present research was to study the effect of the soil herbicides *Terbacil, Pendimethalin* and *Napropamide* on the isolated *Prunus cerasifera* embryos, peach rootstock GF677, apple rootstock MM106, Rootstock *Prunus domestica* "Wangenheims" under *in vitro* conditions.

MATHERIAL AND METHODS

The experiments were carried out in 2001-2009 in the plant biotechnological lab of the Fruit Growing institute – Plovdiv. In vitro isolated *Prunus cerasifera* embryos as well as micropropagated plants without roots and with 10 mm long roots from vegetative peach rootstock GF677, apple rootstock MM106 and rootstock *Prunus domestica* "Wangenheims" were used in the studies. In vitro plants were treated with the soil herbicides *Terbacil* (Sinbar 80 WP) – 100-150 g/da; *Pendimethalin* (Stomp 33 EC) – 400-600 ml/da and *Napropamide* (Devrinol 4 F) – 400-600 ml/da. The herbicide solution (5 ml/cultivation plate-recalculated according to the surface of the *in vitro* used cultural vessels) was laid as a film on the surface of the nutrient medium. Distillated water was used as a control (Rankova et al., 2006 a,b; 2009).

The *in vitro* plants were cultivated in a growth chamber at a temperature of 22±2°C and a photoperiod of 16/8 hours (40 μmol $m^{-2}s^{-1}$ PPFD).

Visual observations on the development and manifestation of external symptoms of phytotoxicity (chlorosis, necrosis, plant withering) were carried out in dynamics on the 7th, 14th and 21st day after the date of treatment. On the 21st day the following biometric indices were reported – plant height (mm), mean number of roots per plant, mean length of the roots (mm), and relative growth rate per plant ($RGR = (\ln FW_{final} - \ln FW_{initial})/21days$), as well as the content of plastid pigments.

RESULTS AND DISCUSSION

Prunus cerasifera L. embryos treated with pendimethalin

Phytotoxicity (inhibition of root meristem growth and browning of cotyledons) was established in the treatment of embryos with embryonic roots< 5mm in length. The embryos whose embryonic roots at the moment of herbicide application were longer than 5 mm did not show any symptoms of phytotoxicity (Gercheva et al., 2002).

Herbicide treatment of peach rootstock GF677 *in vitro*

Visual characteristics of phytotoxicity in the leaves and stems of rooted plants of GF677 under *in vitro* conditions were not established after treatment with the soil herbicides terbacil, pendimethalin and napropamid. These herbicides did not exert an effect on rooting and stem growth of plantlets but caused phytotoxicity expressed in suppression of the root growth. It was most strongly expressed after treatment with napropamid at the two applied rates (Rankova et al., 2004).

Apple rootstock MM106

In both variants with napropamid applied the appearance of necrosis in the root tips was observed on the 7th day. It was established that pendimethalin and napropamid depressed the stem and root growth of the treated plants (Fig.1.). The inhibiting effect of napropamid on those characteristics was expressed even more strongly. Both soil herbicides did not exert any significant effect on the mean number of roots per plant. The application of those herbicides was the reason for the lower content of leaf pigments (chlorophyll *a, в, (a+в)* and carotenoids), the strongest depressing effect being reported after treatment with napropamid (Rankova et al., 2009).

Rootstock *Prunus domestica* "Wangenheims"

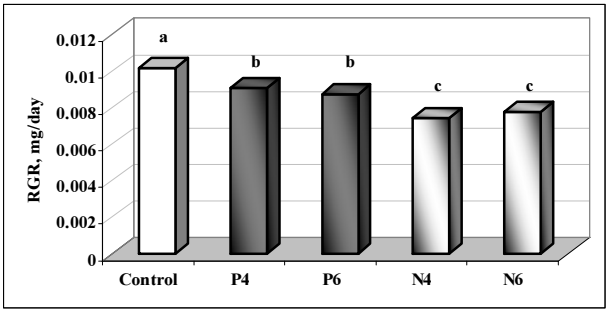
A. Treatment with soil herbicides *Terbacil* (Sinbar 80 WP)

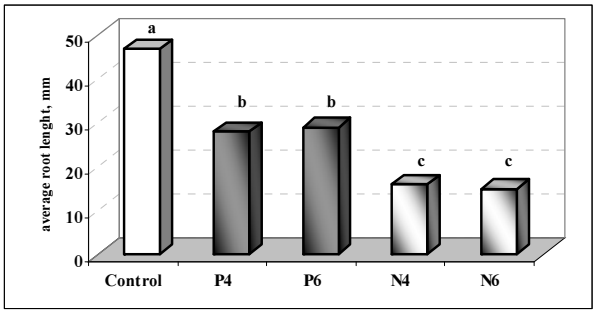
After treatment with terbacil of in vitro rooted plants no external symptoms of phytotoxicity – chlorosis, necrosis and depressing effect were observed. No inhibiting influence on growth of stem and roots were established (Rankova et al., 2006b).

B. Treatment with Pendimethalin and Napropamide

Rooted plantlets

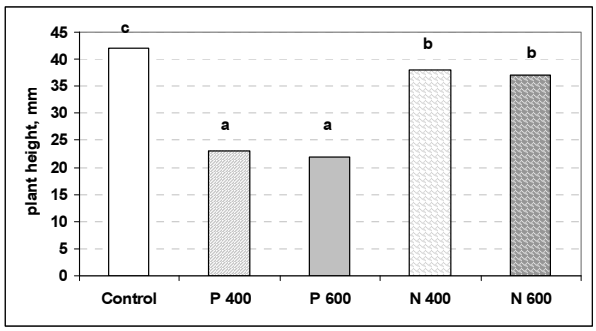
On the 14th day after the application of napropamide on the rooted "Wangenheims" external

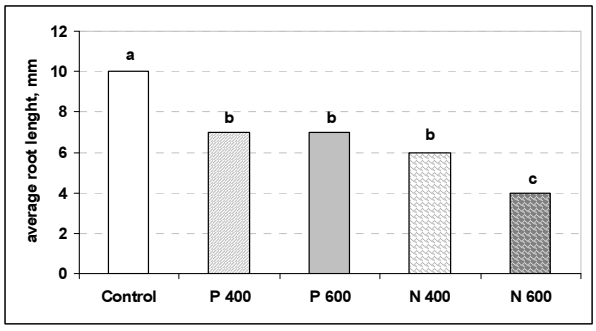




Фиг. 1. *Влияние на почвените хербициди пендиметалин и напропамид върху относителната скорост на растеж (RGR) и дължината на корените на in vitro вкоренени растения от ябълковата подложка ММ106.* ***P4*** *– Pendimethalin – 4.0 l/ha;* ***P6*** *- Pendimethalin – 6.0 l/ha;* ***N4*** *- Napropamid – 4.0 l/ha;* ***N6*** *– Napropamid - 6.0 l/ha. Различните букви на всяка колона показват съществена разлика по Дънкан (DMRT) (P<0.05)*

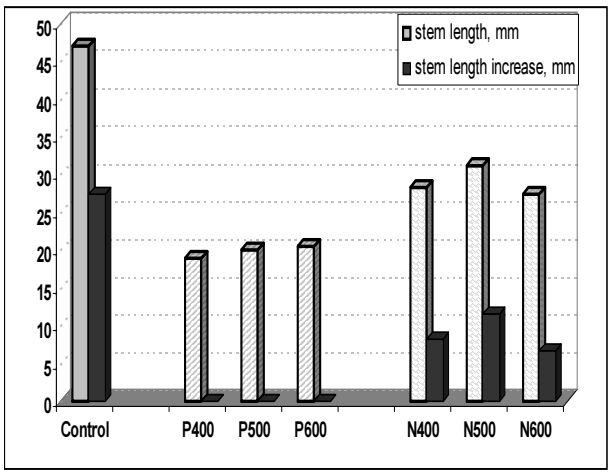
Fig. 1. *Effect of the soil herbicides Pendimethalin and Napropamid on the relative growth rate (RGR) and root length of in vitro rooted apple rootstock MM106.* ***P4*** *– Pendimethalin – 4.0 l/ha;* ***P6*** *- Pendimethalin – 6.0 l/ha;* ***N4*** *- Napropamid – 4.0 l/ha;* ***N6*** *– Napropamid - 6.0 l/ha. Different letters within each column indicates significant difference (P<0.05) by DMRT*





Фиг. 2. *Влияние на почвените хербициди пендиметалин и напропамид върху височината на растенията и дължината на корените на in vitro вкоренени растения от Prunus domestica "Wangenheims".* ***Control*** *(нетретирани);* ***P400*** *– Pendimethalin – 4 l/ha;* ***P600*** *- Pendimethalin – 6 l/ha;* ***N400*** *- Napropamid – 4.0 l/ha; 5.* ***N600*** *– Napropamid - 6 l/ha. Различните букви на всяка колона показват съществена разлика по Дънкан (DMRT) (P<0.05)*

Fig. 2*. Effect of the soil herbicides Pendimethalin and Napropamid on the plant height (mm) and root length (mm) of in vitro rooted Prunus domestica "Wangenheims".* ***Control*** *(untreated);* ***P400*** *– Pendimethalin – 4 l/ha;* ***P600*** *- Pendimethalin – 6 l/ha;* ***N400*** *- Napropamid – 4.0 l/ha; 5.* ***N600*** *– Napropamid - 6 l/ha. Different letters within each column indicates significant difference (P<0.05) by DMRT*



Фиг. 3. *Влияние на почвените хербициди пендиметалин и напропамид върху дължината на стъблата (mm) и прираста (mm) на невкоренени растения от Prunus domestica "Wangenheims".* ***Control*** *(нетретирани);* ***P400, P500, P600*** *– третирани с пендиметалин (съответно 400 ml/da, 500 ml/da и 600 ml/da);* ***P400, P500, P600*** *– третирани с напропамид (съответно 400 ml/da, 500 ml/da и 600 ml/da)*

Fig. 3*. Effect of the soil herbicides Pendimethalin and Napropamid on the stem length (mm) and stem length increase (mm) of Prunus domestica "Wangenheims" plantlets (without roots).* ***Control*** *(untreated);* ***P400, P500, P600*** *- treated with Pendimethalin (400 ml/da, 500 ml/da and 600 ml/da respectively);* ***P400, P500, P600*** *– treated with – Napropamid (400 ml/da, 500 ml/da and 600 ml/da respectively)*

symptoms of phytotoxicity and necrosis in the root formation area were appeared. These symptoms were more pronounced at the higher concentration of napropamide. After treatment with pendimethalin, a chlorosis in the leaves of the plantlets and obvious depression of stem and root growth were reported (Fig. 2).

Plantlets without roots

The soil herbicides pendimethalin and napropamide blocked rooting of *Prunus domestica* "Wangenheims" under *in vitro* conditions. After treatment external symptoms of phytotoxicity and depressing effect on root formation and growth of microplants without roots were established (Fig.3). On the 14th day after the application of napropamide a necrosis in the root formation area was appeared. After treatment with pendimethalin, chlorosis in the leaves of the plantlets and obvious depression of growth were reported (Rankova et al., 2006a).

CONCLUSIONS

The obtained results about influence of the soil herbicides on the growth of rootstocks *in vitro* showed that *in vitro* plants could be an useful model system for evaluation of fruit plants stress responses to soil herbicide treatment. It is necessary an individual approach to different rootstocks – seed and vegetative – depending on the mechanism of phytotoxic action of the active substances.

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