Growth Habits of the Vegetative Apple Rootstock MM 106 after Treatment with Some Soil Herbicides under In Vitro Conditions

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Abstract

The strong susceptibility of the fruit species used as rootstocks to herbicide application necessitated the studies on the vegetative habits and physiological status of the plants with the aim of optimizing the weed control in the production of certified fruit planting material.

The aim of the present study was to develop an in vitro test system for preliminary screening of the effect of some soil herbicides on the growth habits of vegetative apple rootstocks.

Micropropagated and rooted plants of apple rootstock MM 106 (root length about 10 mm) were treated with the soil herbicides pendimethalin and napropamid. The following variants were set for the experiment: 1. Control (untreated); 2. Pendimethalin – Stomp 33 EC – 4.0 L/ha; 3. Pendimethalin – Stomp 33 EC – 6.0 L/ha; 4. Napropamid – Devrinol 4F – 4.0 L/ha; 5. Napropamid – Devrinol 4F – 4.0 L/ha. The rates of the respective herbicides were recalculated according to the area of the cultural vessel.

In both variants with napropamid applied appearance of necrosis in the root tips was observed on the 7th day. On the 14th and 21st days those symptoms were much more expressed and stem growth suppression was also reported.

It was established that pendimethalin and napropamid depressed the stem and root growth of the treated plants. 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, β, a+β* and carotenoids), the strongest depressing effect being reported after treatment with napropamid.

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, 2006). That is why preliminary studies are needed about the effect of different herbicides on the vegetative habits of the rootstocks.

The in vitro plants are a 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). When

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testing the effect of FMX herbicide on growth and photosynthesis of in vitro grapevines, a reduced chlorophyll content in the leaf tissues and a significant decrease of the total biomass, of the photosynthetic gas exchange and the carotenoid content in the leaves were established (Saladin et al., 2003c). The stems and leaves were dehydrated, water and osmotic potential was reduced and accumulation of soluble carbohydrates in all the tissues and of free proline in stems and leaves was detected (Saladin et al., 2003a). Those data presented an obvious indication about the existing stress response of the in vitro cultural plants treated with herbicides. There is a fact of great interest that during in vitro experiments, contrary to data in literature, an effect on cultural plants was established when the herbicide was absorbed through the roots. The results obtained in in vitro screening were confirmed by in vivo observations (Saladin et al., 2003b).

The aim of the present research was to study the effect of the soil herbicides pendimethalin and napropamid on the vegetative habits of microplants of the apple rootstock MM 106 under in vitro conditions.

MATERIALS AND METHODS

The experiments were carried out in 2005-2006 at the Laboratory of Plant Biotechnology of the Fruit Growing Institute – Plovdiv.

In vitro plants of the vegetative apple rootstock MM 106 were propagated on a nutrient medium based on MS (Murashige and Skoog, 1962), enriched with 2,5 μM BAP, 0,005 μM IBA, sucrose 30 g/L, agar 6,5 g/L, pH 5.6 (before autoclaving).

Microplants 2 cm high were set for rooting on the nutrient medium based on MS (25% of macroelements, 100% microelements and vitamins) with added 1,48 μM IBA, 20 g/L sucrose, 6,5 g/L agar, pH 5.6.

Rooted plants having roots about 10 mm long were treated with the soil herbicides. The herbicide solution (recalculated to the surface area of the cultivation plate) was laid as a film over the surface of the nutrient medium.

1. C - Control (untreated) – destillated water - 4.0 L/ha;
2. P4 - Pendimethalin – Stomp 33 EC – 4.0 L/ha;
3. P6 - Pendimethalin – Stomp 33 EC – 6.0 L/ha;
4. N4 - Napropamid - Devrinol 4F – 4.0 L/ha;
5. N6 - Napropamid - Devrinol 4F – 6.0 L/ha.

The in vitro plants were cultivated in a 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 7^{th}, 14^{th} and 21^{st} day after the date of treatment. On the 21^{st} day the following biometric indices were reported – plant height (mm), number of roots per plant in average, mean length of the roots (mm), and relative growth rate per plant (RGR), calculated as $(\ln FW_{final}-\ln FW_{initial})/21days$.

The content of plastid pigments – chlorophyll and carotenoids – in the leaves of the in vitro plants was determined spectrophotometrically in 80% ethanol extract.

Data Analysis

For each variant of herbicide treatment 10 plantlets in four repetitions are set. Data were analyzed by analysis of variance and the means were separated using the Duncan's multiple range test (DMRT) ($P < 0.05$).

RESULTS AND DISCUSSION

The results obtained showed the different effect exerted by the studied soil herbicides on the development of the in vitro plants, expressed in the manifestation of external symptoms of phytotoxicity, as well as in the time those symptoms appeared.

On the 7^{th} day visual symptoms of phytotoxicity in the leaves and stems of the plants were not observed. The apical bud of the explants treated with pendimethalin (variants P4 and P6) and napropamid (variants N4 and N6) was fresh, actively growing, without growth suppression. In both variants with napropamid applied (variants N4 and N6) obviously expressed depression of root growth was observed as well as the appearance of necrosis in their tips.

On the 14th day in both variants treated with pendimethalin (variants P4 and P6) the appearance of slight chlorosis in the leaves, growth suppression and delayed root growth was observed. In the variants treated with napropamid (variants N4 and N6) the vegetation tip was fresh, but slight leaf yellowing and suppression of growth in height were reported. Strongly expressed necrosis in the roots and visible depression of their growth was established.

On the 21st day the external symptoms of phytotoxicity were analogous with those of the 14th day but more obviously expressed.

The results of the biometric analysis showed the different effect of the applied herbicides on the habits of the microplants.

Both herbicides applied suppressed the stem growth in height (Fig. 1). The suppressing effect on growth was most strongly expressed when the higher rate of pendimethalin was applied (variant P6). The values of the plant height were equal when applying the lower rates of both herbicides (variants P4 and N4). That plant response could be explained by the mechanism of action of the active substances and their effect on growth of susceptible plants (Tonev, 2000).

A statistically significant effect of the herbicides on the mean number of roots per plant was not established (Fig. 2). Consequently, the application of pendimethalin and napropamid did not affect the formation of new roots in the microplants.

The results showed a strong suppressing effect of the herbicides applied on root growth (Fig. 3). The lowest values of the root length were reported after treatment with the higher rate of napropamid (variant N6). When applying both rates of the active substance pendimethalin (variants P4 and P6), depression of root growth was also established but the inhibiting effect of pendimethalin was expressed more slightly in comparison with that of napropamid. It could be explained again by the mechanism of phytotoxic action of the active substances and the effect of napropamid on root growth in susceptible species (Tonev, 2000).

The applied herbicides suppressed the growth of the biomass of the plants. The suppressing effect was most strongly expressed when napropamid was applied (variant N4 and N6). The relative growth rate (RGR) of the plants is about 23 to 26% lower than in untreated plants (Fig. 4).

Data about the content of light harvesting pigments in the plant leaves confirmed the depressing effect exerted by pendimethalin and napropamid on plant growth. In all the variants treated with herbicides, a lower content of chlorophyll (*a, β* and *a+β*) and carotenoids was reported (Table 1).

The results obtained gave the grounds to conclude that treatments of the plants of the apple rootstock MM 106 with the herbicides napropamid and pendimethalin at the initial stage after rooting had a negative effect on their growth and development. At a direct contact with the growing roots, the herbicides exerted a strong phytotoxic effect expressed in root growth suppression.

CONCLUSIONS

The soil herbicides pendimethalin and napropamid manifested a strong depressing effect on growth of the apple rootstock MM 106 under in vitro conditions.

Based on the results obtained, it can be concluded that on very light soils and at shallow planting, the application of those two herbicides is risky for inducing strong phytotoxicity in that rootstock.

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<u>Tables</u>

Table 1. Content of light harvesting pigments in the leaves of apple rootstock MM 106 plantlets after treatment with some soil herbicides.

Variants	Pigment content (mg/gDW)			
	Chl a	*Chlβ*	*Chl(a+β)*	carotenoids
Control	3,90	1,51	5,44	0,34
P4	1,74	0,84	2,58	0,19
P6	1,48	0,71	2,19	0,16
N4	2,61	1,09	3,71	0,25
N5	2,51	1,32	3,83	0,24

Figures

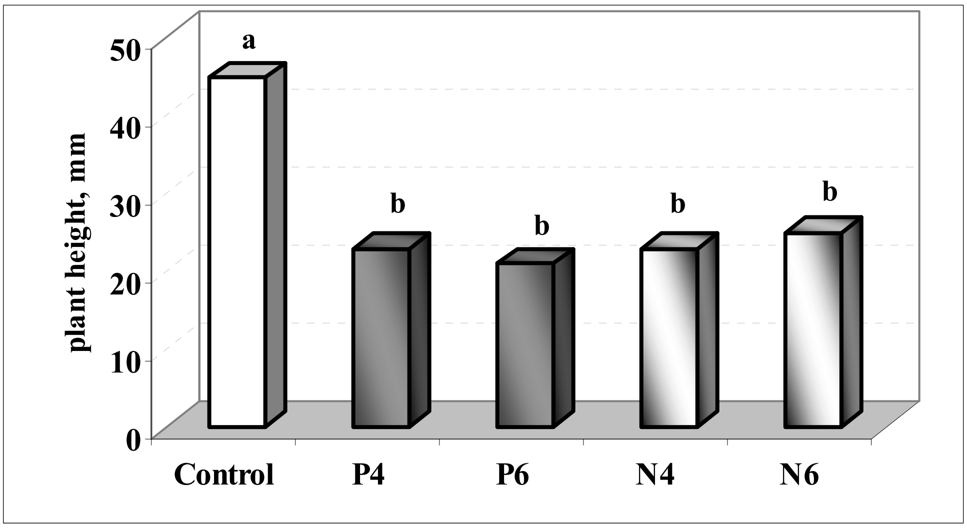

Fig. 1. Effect of the soil herbicides on plant height (mm) 21 days after treatment. Different letters within each column indicate significant difference (P<0.05) by DMRT.

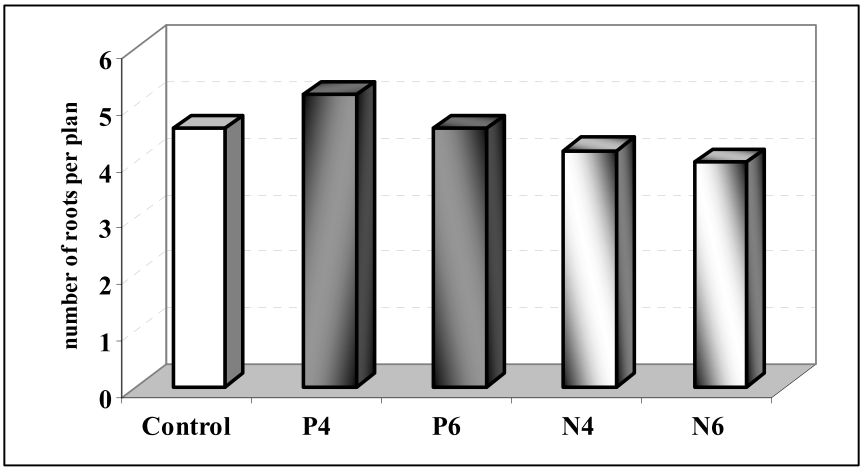

Fig. 2. Effect of the soil herbicides on the number of roots per plant - n.s.

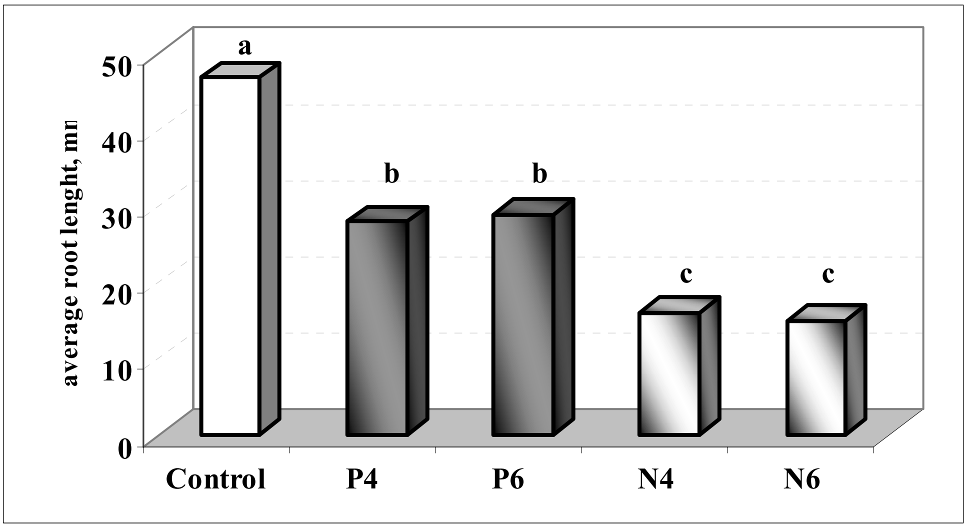

Fig. 3. Effect of the soil herbicides on root length – in average per plant (mm). Different letters within each column indicate significant difference (P<0.05) by DMRT.

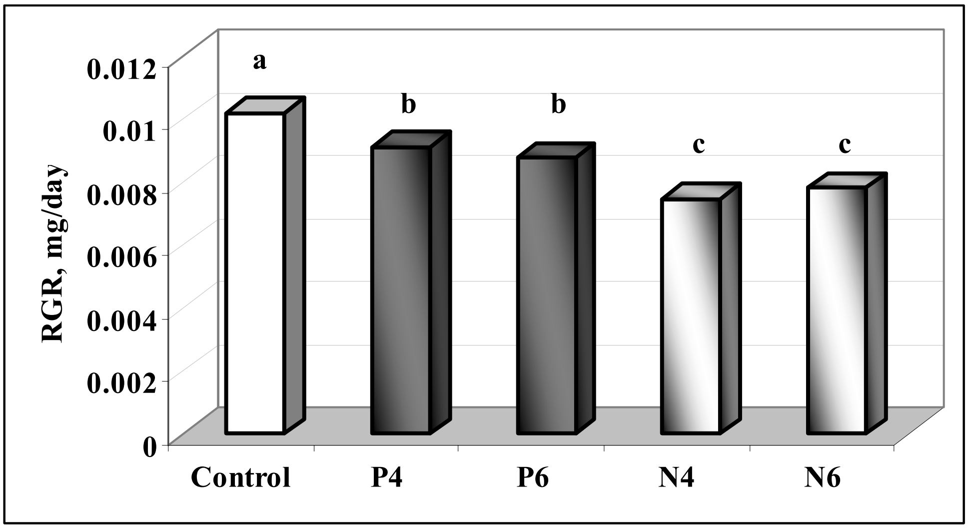

Fig. 4. Effect of the soil herbicides on the relative growth rate (RGR) of the plants. Different letters within each column indicates significant difference (P<0.05) by DMRT.