

## RESPONSE OF *Fuchsia hybrida* CUTTINGS TO FLURPRIMIDOL AND NAPHTHALENEACETIC ACID APPLICATION

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### Abstract

Auxins are the only compounds used in commercial plant propagation to stimulate rooting, although efforts have been made to find other efficient compounds. Another group of rooting promoters could be plant growth retardants (PGRs) which act as inhibitors of gibberellin synthesis. The aim of the experiment was to determine the effect of flurprimidol and napthalene-1-acetic acid (NAA) applied by quick-dip method on rooting and development of *Fuchsia hybrida* ‘Swingtime’ cuttings and their subsequent growth. Anatomical analysis of the rooting process was also done. Flurprimidol and NAA did not influence the percentage of rooted fuchsia cuttings but increased the number of adventitious roots. Both compounds were involved in earlier formation of root primordia. Flurprimidol and NAA, used simultaneously or separately, increased the number and length of axillary shoots of fuchsia cuttings. The effect of flurprimidol on the number of roots and shoots was stronger than that of NAA, but diminished after transplanting the rooted cuttings. The influence of flurprimidol on axillary shoot length was weaker as its concentration increased.

**Key words:** growth retardants, auxins, adventitious roots, anatomical changes, axillary shoots

### INTRODUCTION

The economical success of ornamental horticulture depends on production of plants of the highest quality in the shortest time of production. Growth regulators are commonly used to accelerate propagation and create required plant shape. Until now, auxins have been the main promoters of rooting of cuttings (Hartmann et al. 2002), although there have been made efforts to find other efficient compounds. Another group of compounds stimulating rooting are plant growth retardants (PGRs) (Davis and Sankhla, 1988), but they are not commercially used for this pur-

pose. They act as inhibitors of gibberellin synthesis, thus suppress elongation of shoot internodes. In floriculture, retardants that compromise a nitrogen-containing heterocycle are the most commonly used, such as pyrimidines: ancymidol and flurprimidol, or some triazole-compounds such as paclobutrazol and uniconazole-P (Rademacher, 2000). PGRs deactivate monooxygenases catalyzing oxidation of numerous metabolic pathways. Hence, apart from blocking gibberellin synthesis, PGRs influence the synthesis of sterols and abscisic acid (Jankiewicz, 1997). At the histological level of plant development, the effect of PGRs results from their impact on subapical shoot meristems which are responsible for internode elongation. According to Grossmann (1990), their lower concentrations inhibit cell elongation in this area, whereas the higher ones also suppress cell divisions. Apart from PGR concentration, their effectiveness depends on a number of factors, including the method of application, their persistence and plant response.

PGRs can be applied in many ways. In commercial cultivation, the most popular form of application is foliar spray or substrate drench (Schuch, 1994). Foliar application usually requires higher doses and thus is related to a larger volume of spray solution, which is not of a neutral effect on the environment. Soil drench may have an excessive impact on plants, resulting in too strong height suppression and delayed development because of PGR survival in the substrate (Hwang et al. 2008). Because of the need to reduce the influence of retardant effect on the environment, efforts have been made to find an alternative method of application that would allow to control the amount of the compound applied. Research on PGR-affecting rooting conducted so far has involved soaking or dipping unrooted cuttings as well as a dipping root

system of young plants (Schuch, 1994; Gent, 2004; Wang et al. 2008). These treatments provide a thorough coverage of submerged plant parts. Other methods included the following: encapsulated controlled-release systems (Wiesman et al. 2002), granular formulation (Bunnell and Cockrem, 2005; Grey et al. 2009) as well as recycled subirrigation supply (Million et al. 2002). Positive results were also obtained after mother plant treatment (Wiesman and Lavee, 1994).

Apart from determining plant shape, PGRs exhibit numerous side effects involved in plant quality, such as enhanced branching, accelerated flowering, increased chlorophyll content in leaves, and delayed aging (Hamid and Williams, 1997; Abdulla et al. 1998; Kozak and Grodek, 2005; Yadav et al. 2005; Wang et al. 2008; Nizam and Techato, 2009). Moreover, they cause an increase in the amount of mechanical tissues, which makes plants less susceptible to lodging. Some physiological responses to PGRs, such as increased tolerance to stress factors (e.g. drought, frost and some fungus infections), reduced transpiration and water absorption enhance the survival of plants after transplanting (Premachandra et al. 1997; Kozak, 2006). Improved establishment after PGR treatment may also result from increased rhizogenesis (Abdi and Ascaria-Raburi, 2009; Nizam and Techato, 2009).

Flurprimidol, investigated in the research, is widely used to control shoot elongation in cultivation of ornamental plants, such as *Tibouchina* and *Melastoma* of the order Myrtales, the same that fuchsia belongs to (Abdulla et al. 1998; Kozak and Grodek, 2005). It proved to be efficient in promoting adventitious root formation (Burkhardt and Meyer, 1991). Flurprimidol is more effective through stem application than by root uptake. The poorest effectiveness of flurprimidol was shown in the case of foliar application (Bunnell and Cockrem, 2005).

The aim of the research was to determine the effect of flurprimidol and its cooperation with naphthalene-1-acetic acid (NAA) on rooting and development of *Fuchsia hybrida* 'Swingtime' cuttings as well as on subsequent growth of rooted cuttings. Anatomical analysis of changes taking place in cuttings was also undertaken. From the practical point of view, the study was undertaken in order to receive moderately compact and well branched plants in the shortest time of production.

## MATERIALS AND METHODS

The experiment on propagation of *Fuchsia hybrida* 'Swingtime' by cuttings was carried out in the greenhouse of the Department of Horticulture, Wrocław University of Environmental and Life Sciences, Poland, in March 2009 and 2010. Apical stem cuttings,

4 cm in length, were treated with flurprimidol (contained in the commercial preparation Topflor 015 SL), naphthalene-1-acetic acid or flurprimidol with naphthalene-1-acetic acid in the following combinations (in g  $\times$  dm<sup>-3</sup>): flurprimidol: 0 (control cuttings); 0.075; 0.15 and 0.3; NAA: 0.5; 1.0 and 2.0; flurprimidol + NAA: 0.075 + 1.0; 0.15 + 0.5.

Both compounds were applied by the quick-dip method: the basal ends of cuttings were dipped in the solutions for 5 second before placing in a rooting medium. The medium consisted of white peat, pine bark and perlite 3:1:1; V:V:V, pH 6.4. It was heated to a temperature of 21°C. Low plastic tunnels were installed over the cuttings. The experiment was established in one factorial design with 6 replications, with 10 cuttings per each replication. The measurements, including percentage of rooted cuttings, number of roots, height of cuttings, as well as number and length of axillary shoots, were taken after 4 weeks of rooting. They were done in 3 replications for every cutting. Then, intact cuttings, not exposed to measurement, were planted into pots in peat substrate of pH 6.47 containing (in mg  $\times$  dm<sup>-3</sup>): N-NO<sub>3</sub><sup>-</sup> 145, P 119, K 263, Mg 90, Ca 1120, and placed in a non-heated glasshouse. The experiment was established in 4 replications, with 5 plants per each replication. After 4 weeks of cultivation, the height of plants as well as the number and length of axillary shoots were measured.

The data of the study were subjected to analysis of variance, and the least significant differences between means were calculated by Tukey's test at p = 0.05. The data concerning the percentage of rooted cuttings were first transformed according to Bliss function.

**Anatomical analysis.** For anatomical studies, additional cuttings of *Fuchsia hybrida* 'Swingtime' were prepared. They were treated with the following formulations (in g dm<sup>-3</sup>) applied by quick-dip method: flurprimidol: 0.075; 0.15; NAA 1.0. The last treatment was the control containing untreated cuttings. All cuttings were rooted in the conditions described above. During the first week after placing cuttings in the medium, cutting samples were collected for analysis every 24 hours, whereas subsequent collections took place every 2 days. Transactional sections were made from the basal part of cuttings, 0.5–1cm long. Stem segments were embedded in paraffin, cut into 10 µm sections using a microtome with disposable blades (Boeckeler MR2), stained with acid fuchsin and fast green and covered with Canadian balm. Microscopic analyses were performed under an optical microscope and photographed.

## RESULTS

Regardless of the year of the experiment, no effect of flurprimidol and naphthaleneacetic acid on

percentage of rooted cuttings, applied separately or together, was found. The only inhibitory effect could be observed after the application of NAA at the highest concentration during the second year of experiment. Both growth regulators did increase the number of adventitious roots in both years of research, but the influence of flurprimidol was stronger (Table 1). Regardless of the year of the experiment, flurprimidol at its highest concentration of  $0.3 \text{ g dm}^{-3}$  increased the root number by 250% as compared to control cuttings. There was no significant difference between the effect of flurprimidol and flurprimidol combined with NAA.

The anatomical analysis indicated that the process of root formation in cutting shoots treated with flurprimidol occurred faster when it was administrated at the higher concentration and flurprimidol-induced rhizogenesis was only slightly delayed in comparison to NAA-determined changes. In shoots treated with flurprimidol at  $0.075 \text{ g dm}^{-3}$ , the first primordia emerged after 6 days of rooting (Fig. 1), and after 5 days as a result of flurprimidol application at  $0.15 \text{ g dm}^{-3}$  (Fig. 2), whereas the same stage of primordium formation in NAA-treated cuttings was observed after 4 days (Fig. 3). Apparently, formed root meristems could be visible after 9, 7 and 5 days, respectively. The same stage of root primordia development in control cuttings was observed after 11 days of rooting. Root outgrowth through cutting cortex and getting outside took place after 11 days (Fig. 4), regardless of flurprimidol concentration, compared to 15 days for control cuttings (Fig. 5). Flurprimidol also increased the number

of primordia in cuttings' shoots. In shoots treated with flurprimidol, 5 primordia appeared (Fig. 6), the same number as in shoots treated with auxin, while only up to 3 primordia were observed in control cuttings.

No effect of flurprimidol in lower concentrations on the height of fuchsia cuttings was observed. Only application of flurprimidol at a concentration of  $0.3 \text{ g dm}^{-3}$  inhibited the growth of the main shoot, while a synergistic influence of flurprimidol in both combinations introduced with NAA on suppression of cutting growth was recorded (Table 2). Our experiment showed the influence of both compounds on axillary shoot outgrowth. Flurprimidol and NAA, used separately, increased the number and length of axillary shoots, but the effect of retardant, applied alone or together with auxin, proved to be more advantageous (Table 2). This beneficial effect diminished after transplanting the rooted cuttings. The only treatment stimulating branching of fuchsia plants was flurprimidol at a concentration of  $0.075 \text{ g dm}^{-3}$ . The same treatment had the strongest effect on lateral shoot elongation. In fact, the positive influence of flurprimidol on the sum of axillary shoot length was weaker as its concentration increased (Table 3). The other treatment stimulating shoot growth was flurprimidol at a concentration of  $0.075 \text{ g dm}^{-3}$  combined with NAA  $1.0 \text{ g dm}^{-3}$ . Irrespective of the year of the experiment, flurprimidol decreased the height of plants as compared to both control plants and the ones treated with NAA. Such effect resulted mainly from its influence in the second year of research.

Table 1  
The influence of flurprimidol and NAA on rooting of cuttings of *Fuchsia hybrida* 'Swingtime'

Treatment	Concentration ( $\text{g dm}^{-3}$ )	Feature					
		Rooting* (%)			Number of roots (no. $\times$ cutting $^{-1}$ )		
		2009	2010	Mean	2009	2010	Mean
Control	---	81.4	77.7	<b>79.6</b>	4.9	5.5	<b>5.2</b>
	0.075	76.9	77.7	<b>77.3</b>	11.7	9.7	<b>10.7</b>
Flurprimidol	0.15	90.0	68.9	<b>79.5</b>	11.2	9.9	<b>10.6</b>
	0.3	83.9	66.6	<b>75.3</b>	12.5	13.7	<b>13.1</b>
	0.5	90.0	75.0	<b>82.5</b>	11.0	7.3	<b>9.2</b>
NAA	1.0	90.0	71.6	<b>80.8</b>	7.1	6.7	<b>7.0</b>
	2.0	90.0	61.2	<b>75.6</b>	10.9	7.8	<b>9.4</b>
Flurprimidol +	0.075 + 1.0	90.0	71.6	<b>80.8</b>	12.4	12.4	<b>12.4</b>
NAA	0.15 + 0.5	83.9	63.9	<b>73.9</b>	10.6	9.2	<b>9.9</b>
LSD for treatment		10.1			2.4		
for treatment x year		14.3			3.4		

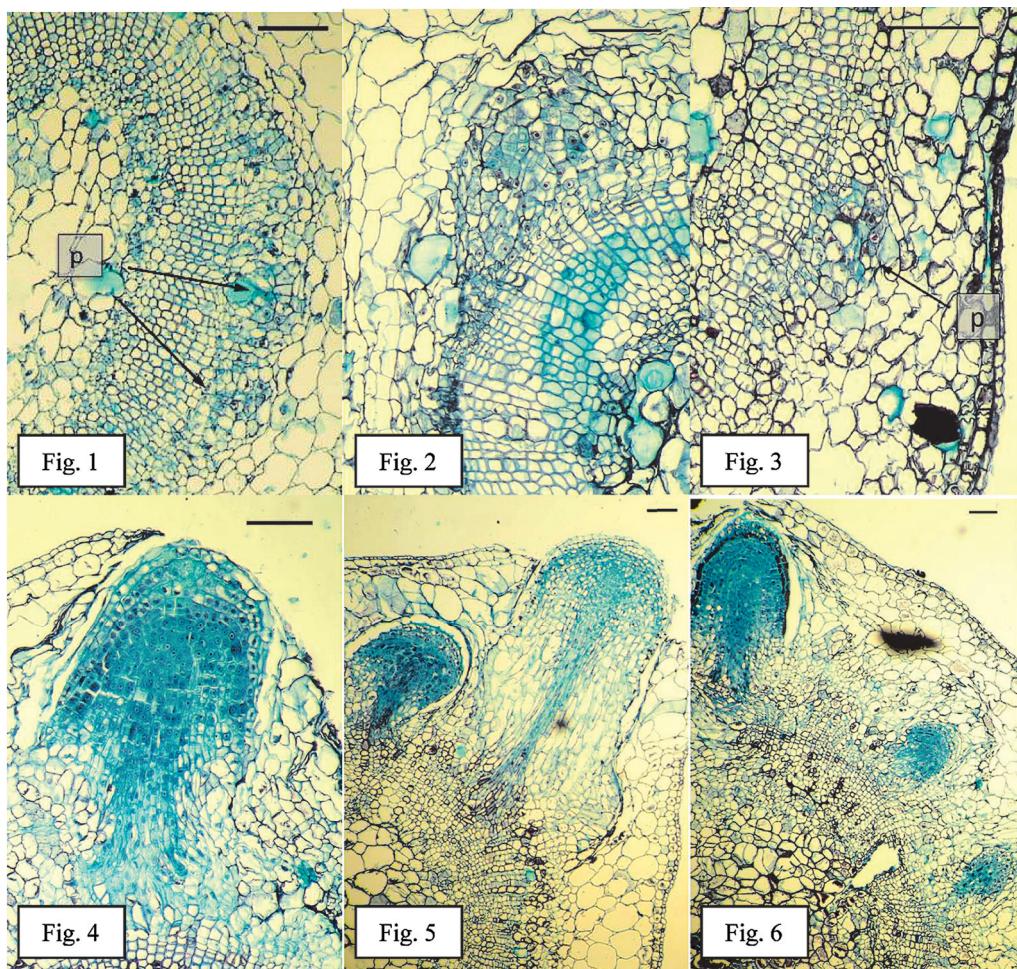
\* Data modified according to Bliss function

Table 2  
The influence of flurprimidol and NAA on shoot development  
in cuttings of *Fuchsia hybrida* 'Swingtime'

Treatment	Concentration (g dm <sup>-3</sup> )	Feature								
		Cutting height (mm)			Number of lateral shoots (no. × cutting <sup>-1</sup> )			Sum of lateral shoot length (mm)		
		2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
Control	---	98.0	60.7	<b>79.3</b>	6.3	1.5	<b>3.9</b>	13.6	5.2	<b>9.4</b>
	0.075	93.1	53.0	<b>73.1</b>	7.7	4.1	<b>5.9</b>	29.2	9.9	<b>19.6</b>
Flurprimidol	0.15	92.1	61.1	<b>76.6</b>	7.4	2.4	<b>4.9</b>	28.8	6.3	<b>17.6</b>
	0.3	75.4	48.1	<b>61.8</b>	8.5	4.2	<b>6.4</b>	30.1	7.2	<b>18.7</b>
	0.5	102.7	70.8	<b>86.8</b>	8.3	2.7	<b>5.5</b>	21.7	6.9	<b>14.3</b>
NAA	1.0	88.9	67.6	<b>78.3</b>	6.4	2.6	<b>4.5</b>	24.2	6.7	<b>15.5</b>
	2.0	89.4	49.3	<b>69.4</b>	8.4	1.4	<b>4.9</b>	24.6	5.2	<b>14.9</b>
Flurprimidol + NAA	0.075 + 1.0	66.1	52.0	<b>59.1</b>	7.9	3.5	<b>5.7</b>	28.6	8.0	<b>18.3</b>
	0.15 + 0.5	61.8	39.4	<b>50.6</b>	7.7	2.6	<b>5.2</b>	30.5	4.0	<b>17.3</b>
LSD for treatment for treatment x year			7.8			0.9			4.0	
			11.0			1.3			5.6	

Table 3  
The influence of flurprimidol and NAA application on cuttings  
on the subsequent growth of young plants of *Fuchsia hybrida* 'Swingtime'

Treatment	Concentration (g dm <sup>-3</sup> )	Feature								
		Cutting height (mm)			Number of lateral shoots (no. × plant <sup>-1</sup> )			Sum of lateral shoot length (mm)		
		2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
Control	---	41.1	90.5	<b>65.8</b>	13.7	7.5	<b>10.6</b>	190.6	51.2	<b>120.9</b>
	0.075	32.7	68.1	<b>50.4</b>	14.8	9.3	<b>12.1</b>	331.3	104.6	<b>218.0</b>
Flurprimidol	0.15	34.6	40.0	<b>37.3</b>	13.7	5.2	<b>9.5</b>	319.5	69.8	<b>194.7</b>
	0.3	27.4	28.9	<b>28.2</b>	11.6	6.2	<b>8.9</b>	289.7	26.3	<b>158.0</b>
	0.5	40.0	104.0	<b>72.0</b>	12.6	6.8	<b>9.9</b>	139.9	50.1	<b>95.0</b>
NAA	1.0	40.7	90.5	<b>65.6</b>	13.0	6.4	<b>9.7</b>	199.5	35.8	<b>117.7</b>
	2.0	46.7	83.4	<b>65.1</b>	14.3	6.8	<b>10.6</b>	200.4	52.0	<b>126.2</b>
Flurprimidol + NAA	0.075 + 1.0	37.8	94.1	<b>66.0</b>	15.0	8.3	<b>11.7</b>	242.7	72.8	<b>157.8</b>
	0.15 + 0.5	31.1	55.8	<b>43.5</b>	13.6	8.2	<b>10.9</b>	217.3	62.7	<b>140.0</b>
LSD for treatment for treatment x year			14.4			1.6			30.5	
			20.4			2.3			43.1	



Figs 1–6. Transverse sections of the shoot of *Fuchsia hybrida* 'Swingtime' cuttings on the following days of rooting: 1. treated with flurprimidol at  $0.075 \text{ g dm}^{-3}$ , day 7, showing adventitious root primordium (p) formation in the cambium and phloem region; 2. treated with flurprimidol at  $0.15 \text{ g dm}^{-3}$ , day 6, showing the formation of an adventitious root primordium; 3. treated with NAA at  $1.0 \text{ g dm}^{-3}$ , day 5, showing cell divisions in an adventitious root primordium (p); 4. treated with flurprimidol at  $0.15 \text{ g dm}^{-3}$ , day 12, showing an adventitious root developing through tissues of the shoot, 5. control, day 16, showing an adventitious root developing through the tissues of the shoot; 6. treated with flurprimidol at  $0.075 \text{ g dm}^{-3}$ , day 7, showing three root primordia. Bars equal 100 µm.

## DISCUSSION

One of hormonal responses to PGRs is a considerable lowering of the level of gibberellins, known for their strong inhibition of rooting. Hence, a decrease in gibberellin content, caused by PGRs, can result in the stimulation of rhizogenesis. The treatment of intact plants with PGR is expressed by an increased root/shoot ratio (Grossmann, 1990), whereas in unrooted cuttings the stimulatory effect on rhizogenesis can be expressed by an increased percentage of rooting as well as the increased number and length of adventitious roots (Darwati et al. 1993; Wiesman and Lavee, 1994; Wang et al. 2008; Kepenek and Karoju, 2011).

Apart from reducing the level of natural gibberellin acid, PGRs also affect the level of other plant

hormones, including auxins and cytokinins, which determine shoot and root architecture. The effect of PGRs on the level of natural auxins is not of a clear-cut significance – they can either increase or decrease natural IAA concentration values in tissues (Pan and Gui, 1997; Shanahan and Soliman, 2011). Nevertheless, numerous researches have proved that it is justified to apply PGRs combined with exogenous auxins. The simultaneous use of both these regulators has proved to be more effective than each of them alone on adventitious root formation in cuttings of many plant species, both herbaceous, like *Phaseolus aureus* L. (Pan and Zhao 1994), and woody, such as *Pinus caribaea* var. *hondurensis* Morelet (Henrique et al. 2006) and *Delonix regia* (Boger.) Raf. (Abdi and Ascarri-Raburi, 2009). The synergistic effect

of growth retardants and exogenous indole-3-butyric acid (IBA) or NAA on rooting has also been observed in *in vitro* cultures (Burkhardt and Meyer, 1991; Wiesman and Lavee, 1994; Nizam and Techato, 2009). The mechanism of this phenomenon has not been unequivocally explained so far. It is believed that the cooperation of these two groups of plant growth regulators can be connected with hormone concentration in particular stages of rhizogenesis. A high concentration of auxins is indispensable, as far as the first stage of rooting, i.e. induction of adventitious roots, is concerned. The reducing effect of gibberellins is revealed in the second stage of root development, in which intensive cell divisions take place. PGRs, as antigibberellins, are probably active in the same stage of rhizogenesis. The positive effects of sequential application of auxins and retardants (IBA followed by the retardant) confirm this hypothesis (Pan and Zhao, 1994). Another explanation of the synergistic effect of auxins and PGRs on rooting can be their stimulatory effect on peroxidase activity, which is responsible for auxin metabolism (Pan and Gui, 1997). This explanation may also be connected with subsequent developmental stages of adventitious root formation, as changes in peroxidase isoform pattern and activity are regarded as biochemical markers of rooting phases (Syrós et al. 2004). Anatomical analysis of *Fuchsia* cuttings corroborates PGR-induced stimulation of primordia formation but it does not allow concluding that there exists any relationship between PGRs and stages of adventitious root development.

Contrary to most of observations focused on PGR and auxin effect on rooting, none of the methods of treatment (PGR alone or with auxin) influenced the percentage of rooting of *Fuchsia* cuttings. Moreover, the cuttings showed a stronger positive response in terms of root number to flurprimidol or NAA comparable to their simultaneous application. Such effect may be associated with a strong PGR influence on cytokinin level in plants. This relation proves, at the same time, to be much more unequivocal than in the case of auxins, as PGR application usually increases the level of endogenous cytokinins in shoot tissues (Jankiewicz, 1997; Rademacher, 2000). It may also occur as a consequence of a decreased level of gibberellins, which act antagonistically on cytokinin activity in apical shoot meristems (Shani et al. 2006). The strong promoting influence of flurprimidol on branching of *Fuchsia* cuttings may also suggest the increase in cytokinin level after PGR application. Cytokinins are responsible for releasing from apical dominance. Many papers have documented that PGR treatment stimulates growth of axillary shoots in some plant species, both *in vivo* and *in vitro* (Hamid and Williams, 1997; Kozak and Grodeck,

2005, Abdelgadir et al. 2009; Kepenek and Karolu, 2011). The poorer effect of flurprimidol on axillary shoot development after transplanting rooted cuttings may be a consequence of single application of this retardant. To obtain plants with abundant tillering, it may be necessary to repeat the application after transplanting (Million et al. 2002).

## CONCLUSIONS

1. Regardless of the year of the experiment, flurprimidol and NAA did not influence the percentage of rooting of *Fuchsia hybrida* 'Swingtime' cuttings, but increased the number of adventitious roots, flurprimidol being more efficient than NAA. Both compounds were involved in the earlier formation of adventitious root primordia.
2. Flurprimidol and NAA, used simultaneously or separately, increased the number and length of axillary shoots in fuchsia cuttings, but the effect of growth retardant was stronger. The only treatment stimulating branching of fuchsia plants was flurprimidol at a concentration of 0.075 g dm<sup>-3</sup>. The positive influence of flurprimidol on the sum of axillary shoot length was weaker as its concentration increased.

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**Reakcja sadzonek  
fuksji ogrodowej *Fuchsia hybrida*  
na fluropirimidol i kwas naftylo-1-octowy**

**S t r e s z c z e n i e**

Auksyny to jedyna grupa związków stosowanych w produkcji roślin w celu stymulowania ukorzeniania, chociaż podejmowane są wysiłki w celu znalezienia innych skutecznych preparatów. Inną grupą stymulatorów ukorzeniania mogą być retardanty wzrostu roślin, których działanie polega na hamowaniu syntezy giberelin. Celem doświadczenia była ocena wpływu fluropirimidolu i kwasu naftylooctowego podawanych

metodą quick-dip na ukorzenianie i rozwój sadzonek fuksji ogrodowej ‘Swingtime’ oraz ich wzrost następczy. Przeprowadzono także analizę anatomiczną procesu ukorzeniania. Fluropirimidol i NAA nie wpłynęły na procent ukorzenienia sadzonek fuksji, zwiększyły natomiast liczbę korzeni przybyszowych na sadzonkach. Obydwa związki przyspieszały tworzenie związków korzeni przybyszowych. Fluropirimidol i NAA, zastosowane razem lub osobno, zwiększały liczbę i długość pędów bocznych na sadzonkach. Wpływ fluropirimidolu na liczbę korzeni i pędów bocznych był silniejszy niż NAA, ale zanikał po przesadzeniu ukorzenionych sadzonek. Wpływ fluropirimidolu na długość pędów bocznych słabł wraz z jego wzrastającym stężeniem.