The effect of fluridone and flurochloridone on the incidence of albinism in pea (*Pisum sativum*) and on the abscission of leaves of privet (*Ligustrum vulgare*)

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ABSTRACT

The effect of fluridone (or flurochloridone), the inhibitor of carotenoid, chlorophyll and abscisic acid synthesis, on the abscission of *Ligustrum vulgare* leaves was investigated. Both forms of fluridone inhibited the abscission of petioles when they were applied as a 1.0% concentration in lanolin to the leaf blade. Fluridone was capable of inhibiting abscission even when it was applied to the petiole as late as 7 hours after the blade was cut off. Fluridone applied in lanolin to the apical part of intact pea seedlings or on the cut surface after decapitation of the epicotyl caused albinism of the stipules. The degree of albinism decreased according to the concentration of applied flurodine (from the highest – 0.5% to the lowest – 0.03%) and was higher in intact than in decapitated plants. Albinism also appeared in pea seedlings grown from seeds swollen in differently concentrated solutions of flurochloridone, particularly in the basal part of the shoots. Complete albinism occurred only in plants cultivated from seeds swollen in high concentrations (0.06–0.12%). The lowest concentration (0.007%) stimulated root growth of the pea seedlings, but inhibited epicotyl growth.

Keywords: fluridone; flurochloridone; abscission; albinism; pea *Pisum sativum*; *Ligustrum vulgare*

Fluridone is an inhibitor of carotenoid, chlorophyll and abscisic acid (ABA) synthesis and was used, in the first place, to shed light on physiological phenomena attributed to ABA. For instance, it was confirmed that due to the effect of ABA seed dormancy was enhanced (Garello and Page-Degivry 1999, Grappin et al. 2000), as well as resistance to drought (Popova 1998, Popova and Riddle 1996), to frost (Yu and Griffith 2001) and salinity (Bellaire et al. 2000). In contrast, by means of fluridone the role of ABA in root gravitropism was not confirmed (Moore 1990). Madhu et al. (1999) studied the correlation between fluridone as an inhibitor of ABA synthesis and abscission but he devoted his attention only to flowers of the cacao tree. The leaves were not given proper attention in spite of the fact that the very name abscisic acid is based on the positive effect of ABA on leaf abscission assumed early by Wareing and Phillips (1978). The first step of our project was to focus on the correlation between various methods of applications of fluridone and leaf abscission.

It was proved that when the plants were treated with fluridone ABA biosynthesis took place after the cleavage of xantophyl to xantoxin, so that fluridone is also the inhibitor of synthesis of both carotenoids and chlorophyll (Gamble and Mullet 1986). The relation of fluridone to this synthesis has been explored, for instance, in rape and sugar beet (Ragolsky and Thorpe 1989), bean (Popova and Riddle 1996), alfalfa (Meyers et al. 1996) and barley (Popova 1998). Our next task was to discover the effect of various methods and forms of application of fluridone on chlorophyll synthesis, seedling growth and on the occurrence of albinism in pea.

MATERIAL AND METHODS

Shoots of *Ligustrum vulgare* with 8 to 10 pairs of leaves giving segments with 1 to 2 leaf pairs in the middle part were cut off in May to June and were used for experiments exploring the rate of leaf abscission. The segments were cultivated under laboratory light at 20 ± 2°C in vessels with water. Lanolin paste with a 0.1% addition of fluridone (F) ($C_{19}H_{14}F_3NO$, Duchefa, Holland) or flurochloridone (Fch) ($C_{12}H_{10}Cl_2F_3NO$, Syngenta Agrochemicals, Fernhurst, Great Britain) was applied to the leaf blades or petiole stumps. The RACER 25 EC preparation contains a concentration of 250 $g.l^{-1}$ of Fch. In one experiment we used lanolin paste with an addition of 0.1% IAA (indolyl-3-acetic acid, Lachema, Czech Republic).

The rate of abscission of the petiole stumps was statistically interpreted in various time intervals after cutting off the blades.

Pea of the variety Lantra was used for experiments on the occurrence of albinism. Seeds were swollen in water and the seedlings were cultivated in vessels with water under laboratory light. Lanolin pastes containing various concentrations of F were applied either onto intact plants or plants decapitated under the primary scale. Fch was also tested; pea seeds were swollen in solutions containing various Fch concentrations. The occurrence of albinism, the growth of roots and epicotyls of pea seedlings was studied and their length was statistically interpreted. The albinism of stipules was investigated using the epifluorescence microscope.

Table 1. The effect of fluridone on the abscission of four petiole stumps of one *Ligustrum vulgare* segments (application to the blade)

Series	Application	Abscised petiole stumps	
		24 h*	48 h**
		after cutting off the blade	
1	fluridone	0.46 ± 0.73	1.60 ± 1.30
2	IAA	0.20 ± 0.48	0.33 ± 0.71
3	control	1.30 ± 0.83	4.00 ± 0.0

* differences between series 1–3, 2–3, are highly significant, differences between series 1–2 are insignificant
** differences between series 1–3, 2–3 1–2 are highly significant

The experimental plants were selected as conventional model species used in the experimental morphologic school of prof. Dostál. Details of the used methods are given in the descriptions of the respective experiments.

RESULTS

FLURIDONE (F) APPLICATION

The effect of F on *Ligustrum vulgare* leaf abscission

Application to the blade. The experiment was focused on the effect of F applied to the blades on leaf abscission when compared to IAA. Both F and IAA were applied as 0.1% lanolin paste onto the blades, which were cut off after 24 hours; 24 and 48 hours later the abscission of petiole stumps was determined. Compared to control plants, the inhibition of abscission by both F and IAA was highly significant, and 48 hours after cutting off the blade the inhibiting effect of F was highly significantly lower than that of IAA (Table 1).

Application to the petiole stumps. In the next experiment, F was applied to the petiole stumps after cutting off the blades. In this experiment the focus was on how long after cutting off the blade could the application of F still influence the rate of petiole abscission. The F in 0.1% lanolin paste was applied 0.5 to 15 hours after cutting off the blade. If F was applied to the petioles deprived of blades after 0.5 to 7 hours, the abscission caused by fluridone was highly significantly inhibited 22 hours after cutting. However, up to 24 hours after cutting off the blades F inhibited abscission only in the case that it was applied 0.5 hours after cutting off the blade (Table 2).

The effect on the occurrence of albinism in *Pisum sativum*. Different concentrations of F were applied to the apical part of the epicotyl of intact pea seedlings and to the cut surface after decapitation of the epicotyl. Depending on the concentrations, F inhibited chlorophyll synthesis in intact plants, and various degrees of albinism appeared on the plants (Figure 1). In decapitated plants, the occurrence of albino plants was lower (Figure 2).

FLUROCHLORIDONE (Fch) APPLICATION

The effect of Fch on the abscission of *Ligustrum vulgare* leaves

Segments from the middle part of the shoots with two pairs of leaves were divided into apical and basal parts and the blades were treated with 0.1% Fch paste. Four days later, the blades were cut off and 48 hours after defoliation petiole abscission was observed and was compared with the controls (Table 3). The table shows that the inhibition of abscission due to Fch was highly significant only in the basal segment where abscission was quicker than in the apical segment; however, this effect was highly significant only in the control segment.

The effect of swelling *Pisum sativum* seeds in a Fch solution on the occurrence of albinism

The seeds were soaked in 4 concentrations of Fch solutions for 12 hours, the controls in water, and then sown out in vegetation pots with soil. The growth of the pea seedlings is given in Table 4 showing that in 2 and 6-day old plants the 0.0007% concentration of Fch stimulated root growth. All Fch concentrations inhibited the growth of epicotyls of 6-day old plants, but only the highest concentration of 0.03% inhibited root growth. Albinism appeared in 12-day old plants only when this concentration was used, i.e. on the first primary scale and/or on the epicotyl base.

In order to verify the potential occurrence of a higher degree of albinism caused by Fch, the seeds were swollen for 12 hours in 5 concentrations of Fch (0.12, 0.06, 0.03, 0.015, 0.0015%) and in water. After sowing out into vegetation pots with soil, inhibition and total albinism appeared with 0.06–0.12% concentrations. In plants where the 0.03% concentration was used, albinism gradually declined (Figure 3). The epifluorescence microscope was used to compare the completely albinic stipules of fluro-

Table 2. The effect of fluridone on the abscission of two petiole stumps of one *Ligustrum vulgare* segment (application onto stumps)

Hours of application of fluridone on petiole stump after cutting off the blade		0.5	2	4	7	15	Control
Abscised petioles after	22 h	0.42 ± 0.7	0.50 ± 0.7	0.32 ± 0.6	1.05 ± 0.9	1.67 ± 0.7	1.68 ± 0.6
	24 h	1.42 ± 0.8	1.92 ± 0.3	1.85 ± 0.5	1.95 ± 0.3	2.00 ± 0.0	2.00 ± 0.0

Table 3. The effect of flurochloridone on the abscission of *Ligustrum vulgare* petiole stumps

Series	Application	Segment	Abscission from 2 petiole stumps
1	control	apical	0.76 ± 0.78
2		basal	1.41 ± 0.70
3	Fch	apical	0.59 ± 0.78
4		basal	0.87 ± 0.89

Differences between series 2–4, 1–2 are highly significant, differences between series 1–3, 3–4 are insignificant

chloridone-treated plants with stipules of untreated plants (Figure 4).

DISCUSSION

ABA is known to accelerate fruit and leaf abscission (Wareing and Phillips 1978). In the current study, we explored if fluridone as an inhibitor of ABA synthesis is capable of inhibiting leaf abscission. In literature, the inhibiting effect of F on abscission was reported in flowers of the cacao tree (Madhu et al. 1999). In our experiments, both fluridone and flurochloridone were found to be capable of inhibiting the abscission of *Ligustrum vulgare* leaves. If applied to the blades both forms of fluridone inhibited petiole abscission. However, when we compared a 0.1% concentration of F with the same concentration of IAA we saw that within 48 hours after cutting off the blade inhibiting effect of IAA on abscission was 5 times higher than the effect of F. We know that a concentration of 10^{-11}% IAA still inhibits the abscission of *Ligustrum vulgare* leaves (Šebánek and Klíčová 1998).

Fluridone inhibits the synthesis not only of ABA but also of carotenoids and chlorophyll (Popova 1998) and this may result in carotenoid and chlorophyll deficiency (Gamble and Mullet 1986) or even destruction of the inner structure of chloroplasts (Popova and Riddle 1996). The occurrence of albinism after fluridone application was described, for instance, in *Allium wakegi* (Yamazaki et al. 1999), barley (Popova 1998) and alfalfa (Meyers et al. 1996) largely in association with investigations of dormancy or effects of stress. Our experiments proved that when F was applied onto the apical part of intact pea seedlings a higher degree of albinism appeared than when applied to the cut surface after epicotyl decapitation. This means that the effect of F is stronger if applied in the immediate proximity of the meristem tissue of the apical buds than if it has to be basally transported to the meristem of the cotylary axillars. The application of 0.12–0.03% Fch, in which the pea seeds were swollen, is a direct intervention into the embryo, insomuch that albinism starts up from the very beginning of germination and in later stages of development it is evident particularly in the basal parts of the epicotyls. Low concentrations of Fch (0.007%), in which the pea seeds were swollen, have no effect whatsoever on plant albinism, but they are capable of stimulating root growth and inhibiting the growth of epicotyls. In a similar way the anti-gibberellin active growth regulators, for instance, chlorcholinchloride (Kopecký and Šebánek 1970) also cause this growth correlation reversion between the roots and stems. Harvey et al. (1994) described the stimulating effect of fluridone on root growth of potatoes *in vitro*.

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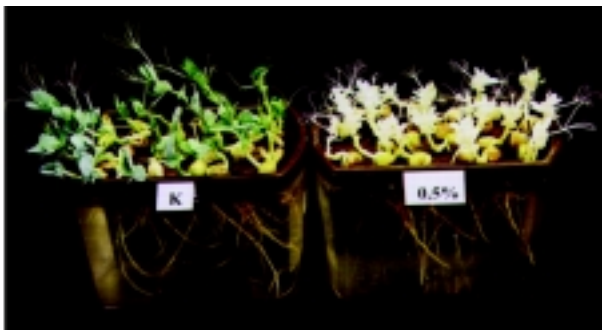
Table 4. Growth of pea seedlings after swelling the seeds in a flurochloridone solution

Series	Fch application	2-day old plants	6-day old plants	
		root length* (mm)	epicotyl length** (mm)	root length*** (mm)
1	0	18.7 ± 4.5	41.7 ± 6.0	84.7 ± 21.6
2	0.0007	23.2 ± 4.5	31.0 ± 6.4	100.3 ± 24.3
3	0.0015	20.3 ± 4.5	36.3 ± 7.1	91.9 ± 17.5
4	0.003	12.6 ± 6.5	32.8 ± 6.9	83.8 ± 19.6
5	0.03	6.0 ± 5.3	23.0 ± 2.7	60.6 ± 14.1

* differences between 1–2, 1–4, 1–5 are highly significant
** differences between 1–3, 1–4, 1–5 are highly significant, differences between 1–2, are significant
*** differences between 1–2, 1–5 are significant



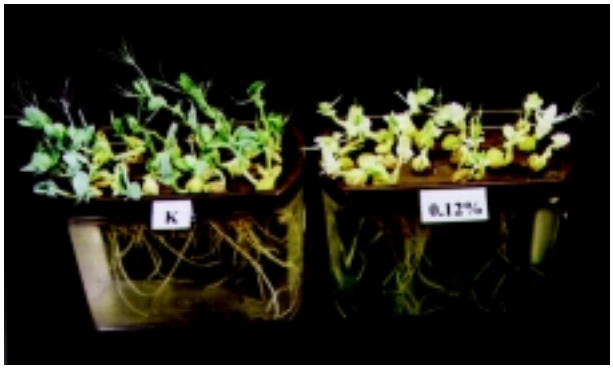
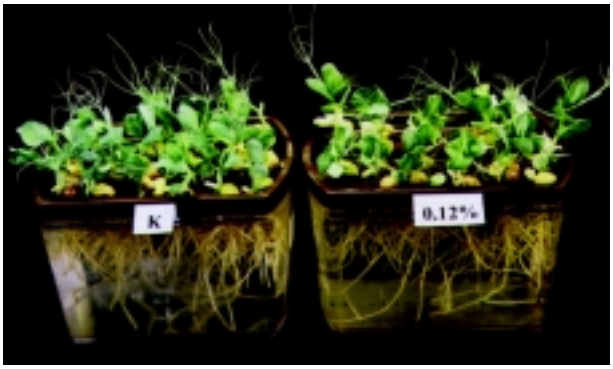


Figure 1. The effect of fluridone on the degree of albinism in intact pea plants; Fluridone 0.5%, 0.12%, 0.03%, K = control



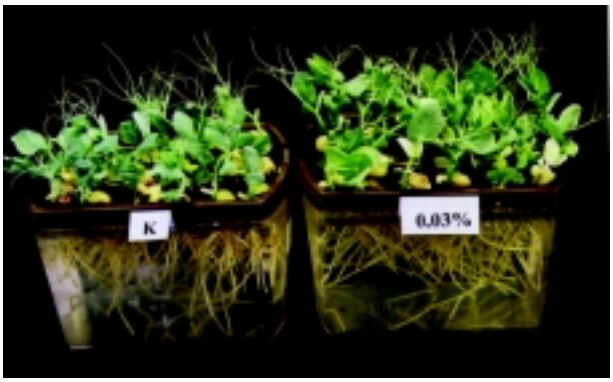


Figure 2. The effect of fluridone on the degree of albinism in decapitated pea plants; Fluridone 0.5%, 0.12%, 0,03%, K = control

Figure 3. From left to right: pea plants germinated from seeds swollen in various concentrations of flurochloridone (0.12, 0.06, 0.03, 0.015 and 0.0015%) and in water

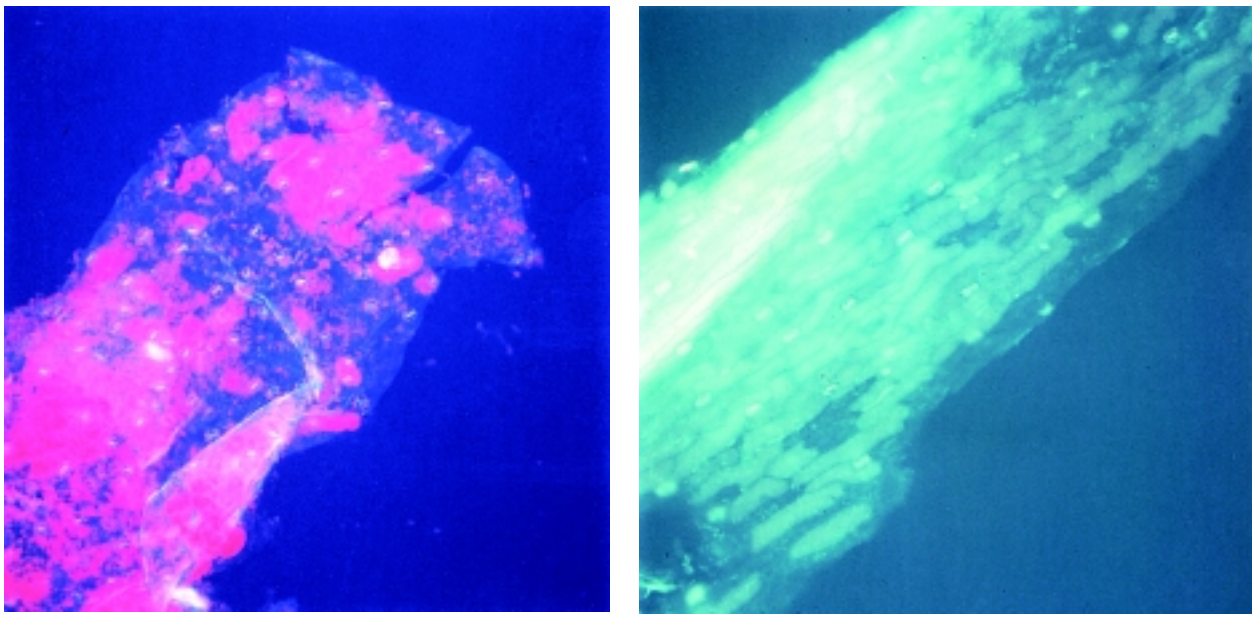


Figure 4. Under an epifluorescence microscope employing blue excitation (460–490 nm) the cells of control stipule were observed as red (left) because of autofluorescence arising from intracellular chlorophyll, whereas stipule of pea treated by 0.06% of fluridon was observed as blue and showed no chlorophyll autofluorescence (right)

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ABSTRAKT

Vliv fluridonu a flurochloridonu na vznik albinismu u hrachu (*Pisum sativum*) a na abscisi listů ptačího zobu (*Ligustrum vulgare*)

Fluridon (popř. flurochloridon), inhibitor syntézy karotenoidů, chlorofylu a abscisové kyseliny, byl použit ke zkoumání vlivu na abscisi listů *Ligustrum vulgare*. Obě formy fluridonu brzdily abscisi řapíků po aplikaci 0,1% koncentrace v lanolinu na čepele. Fluridon byl schopen brzdit abscisi ještě tehdy, byl-li aplikován na řapík i za 7 h po odříznutí čepele. Fluri-

don aplikovaný v lanolinu na vrcholovou část intaktních klíčních rostlin hrachu nebo na řeznou plochu vzniklou po odříznutí jejich epikotylu působil na vznik albinismu palistů. Stupeň tohoto výskytu klesal od nejvyšší (0,5 %) k nejnižší (0,03 %) použité koncentraci fluridonu a byl vyšší u rostlin intaktních než u dekapitovaných. I u klíčních rostlin hrachu vyrostlých ze semen zbobtnaných v různě koncentrovaných roztocích fluridonu byl zřejmý albinismus zejména v bazální části prýtů. Úplný byl tento albinismus jen u rostlin vyrostlých ze semen zbobtnaných ve vysokých koncentracích (0,06–0,12 %). Nejnižší koncentrace (0,0007 %) stimulovala růst kořenů klíčních rostlin, ale inhibovala růst epikotylů.

Klíčová slova: fluridon; flurochloridon; opad; albinismus; hrách *Pisum sativum*; *Ligustrum vulgare*

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