

The effect of quercetine on leaf abscission of apple tree (*Malus domestica* Borkh.), growth of flax (*Linum usitatissimum* L.) and pea (*Pisum sativum* L.), and ethylene production

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ABSTRACT

This study compares effects of 2,3,5-triiodobenzoic acid (TIBA) and flavonoid quercetine (Q). In spite of the fact that both these compounds are inhibitors of the polar transport of auxin, a number of experiments demonstrated that their properties are different. If the flax seedlings were decapitated and one cotyledon was removed, the axillary bud of the remaining cotyledon grew up more intensively while TIBA (0.5% applied in the form of a lanolin paste on the remaining cotyledon) induced a correlative reversal so that axillars of removed cotyledons grew up more intensively. However, when Q (0.5%) was applied on the remaining cotyledon in the form of a lanolin paste, this inhibition was not significant. In the lamina-deprived petioles of apple trees TIBA accelerated abscission while Q caused an inhibiting effect similar to that of auxin. TIBA applied on epicotyls of pea seedlings inhibited their growth by 35% while Q only by 15%. As far as the release of ethylene by pea seedlings is concerned, both compounds showed promoting effects similar to the effect of auxin.

Keywords: IAA; TIBA; *Ligustrum ovalifolium*; *Malus domestica*; growth correlations

The long-distance auxin transport from the stem apex to individual cells is of a strictly polar nature, i.e. to the stem base and to the root tips. On the other hand, auxins are transported from leaves through vascular tissues and their transport does not take place in the polar manner. Polar auxin transport is inhibited above all by 2,3,5-triiodobenzoic acid (TIBA), naphthylphthalamic acid (NPA), and flavonoid quercetine (Q) (Niedergang-Kamien and Skoog 1956, Petrášek et al. 2003, Morris et al. 2004). NPA is bound to a receptor in the plasmalemma and stimulates the accumulation of IAA in the cell through the inhibition of its efflux. Flavonoids compete with NPA for binding sites on the receptor (Macháčková 1997). In plants, the flavonoid quercetine occurs as a native substance. Its high concentrations were found for example in a number of species belonging to the genus *Lathyrus* (Ranabahu and Harborne 1993), in pea shoots and seeds (Ferrerres et al. 1995, Duenas et al. 2004) and in leaves of the species *Ligustrum vulgare* (Romani et al. 2000, Tattini et al. 2004). The fact that Q is a native regulator of auxin transport was demonstrated also in a mutant of *Arabidopsis thaliana* with a low content of

this flavonoid where the auxin transport reached a high intensity (Murphy et al. 2000).

Until now only 2,3,5-triiodobenzoic acid was used in morphogenic studies when the influence of auxin polar transport was examined. These studies were gradually followed by the discovery made by Zimmermann and Hitchcock (1942) who first noticed that this compound markedly accelerated the formation of flowers. Later on, it was found out that TIBA induces a number of other morphogenetic phenomena that were opposite to auxin effects: it inhibited the elongation growth of the stem and promoted its branching, suppressed the growth of adventitious roots, promoted tuber formation, changed the nature of growth correlations in seedlings etc. (Dostál 1967, Šebánek et al. 1991, Procházka et al. 1997). On the other hand, no attention was paid to morphogenetic effects of Q as an inhibitor of polar auxin transport. The objective of this study was therefore to compare the effects of TIBA and Q as inhibitors of polar auxin transport on the abscission and morphogenesis of intact plants with regard to its influence on the synthesis of ethylene that represents an important plant hormone.

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MATERIAL AND METHODS

Experiments with abscission of leaf petioles were performed after the removal of laminae on annual shoots of apple trees (*Malus domestica* Borkh., cv. Juno) and privet (*Ligustrum ovalifolium* Hassk.) that were cut off and immersed with bases into containers with water. Experiments with apple shoots were performed under natural light conditions in laboratory in August, those with privet in September. The growth of pea (*Pisum sativum* L., cv. Oskar) epicotyls was observed after seeding water-soaked seeds into boxes containing garden substrate that were kept in darkness. Laboratory experiments with flax (*Linum usitatissimum* L., cv. Venica) seedlings were performed in daylight during July and August. All experiments were performed at the temperature of $20 \pm 2^\circ\text{C}$. A detailed description of these experiments is presented in the chapter Results. Growth regulators, i.e. IAA, TIBA and Q (all manufactured in LACHEMA, Czech Republic), were 0.5% (w/v) applied in the form of lanolin pastes. Averages and their standard errors are presented in the graphs.

Estimation of ethylene production

Glass jars with the volume of 260 ml containing five 5-day-old pea seedlings in 90 ml of distilled water were placed into plastic stands. Epicotyls of these pea seedlings were treated with a lanolin paste containing 0.5% IAA, TIBA and Q. Metallic caps with a septum enabling to sample gases with a syringe were used to close the jars, which were stored in darkness at the temperature of 21°C . After 60 minutes of cultivation of seedlings in a closed jar, samples of internal atmosphere were taken off and the jars were opened, ventilated and closed again to start the subsequent period of ethylene production. Gas samples were analysed in a gas chromatograph (manufacturer FISSONS INSTRUMENT, Italy) with a 50-meter capillary column Al_2O_3 S 15 μm , ID 0.53 mm. When estimating gaseous hydrocarbons, spray, column, and detector temperatures were 230, 40 and 200°C , respectively (Fišerová et al. 2001).

Statistical evaluations were conducted after the conversion to the ethylene standard in 1 ml of the air from the space where it was taken. The gas contents (7 replications) were averaged and the mean error was calculated.

RESULTS

ABSCISSION OF LAMINA-REMOVED PETIOLES

Experiments with apple tree

Comparison of the effects of Q and IAA. Annual shoots with 13–15 leaves were cut on 28 August 2005 and decapitated in such a way that their apical part with three top leaves was cut off. Only 4 petioles without laminae were left on each annual shoot. All other leaves were removed. The experiment involved altogether 100 petioles. Experimental material was divided into three groups. The first one involved petioles treated with the lanolin paste containing 0.5% IAA on the cut surface, the second one treated with the same paste but with 0.5% Q and the third one served as untreated control (or, in replications with an aqueous lanolin paste). Numbers of separated petioles are presented in Figure 1. Q markedly inhibited the abscission of petioles and this separation was approximately the same as that caused by IAA. At the beginning of the experiment this inhibition was a little weaker but in the final stage it was a little stronger than that caused by IAA.

Comparison of the effects of Q and TIBA. Annual shoots with 13–15 leaves were cut on 26 August 2005 and decapitated in such a way that their apical part with three top leaves was cut off. Only 7 petioles without laminae were left on each annual shoot. All other leaves were removed. The experiment involved 217 petioles altogether. Experimental material was divided into three groups. The first one involved petioles treated on the cut surface with the lanolin paste containing 0.5% TIBA, the second with the same paste but with 0.5 % Q and the third one served as untreated control (or, in replications with an aqueous lanolin paste). Numbers of separated petioles are presented in Figure 2; in this case, however, Q again inhibited the abscission of petioles while TIBA showed a marked accelerating effect.

Experiments with privet (*Ligustrum ovalifolium*)

On 24 September 2005, annual shoots with 10 pairs of leaves were sampled and decapitated to remove the top two pairs of leaves. The next two pairs of leaves whose laminae were cut off and all other leaves were removed. The experiment

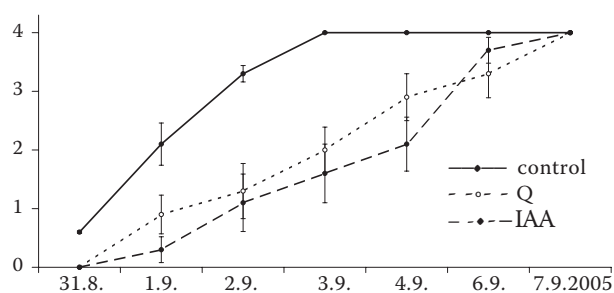


Figure 1. Effect of the lanoline paste containing either 0.5% IAA or Q on the rate of apple petioles abscission as compared with control

y-axis = number of abscised petioles, x-axis = time

involved 80 annual shoots altogether, each with two pairs of petioles. In the first group of shoots, petiole stumps were treated on the cut surface with the lanolin paste containing 0.5% TIBA, in the second with the same paste but with 0.5% Q and the third one was used as untreated control again. As shown in Figure 3, in this case Q showed a marked inhibiting effect on the abscission of petiole stumps; TIBA, however, did not accelerate it but showed an inhibiting effect as well (although not as intensive as Q).

CORRELATIONS BETWEEN COTYLEDONS AND THEIR AXILLARY BUDS IN FLAX

Flax was sown on 27 July 2005 and seedlings were decapitated just above cotyledons after one week

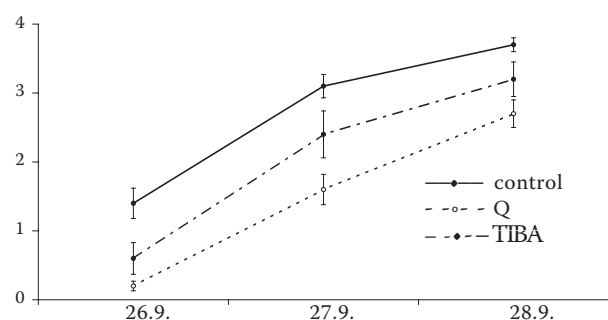


Figure 3. Effect of the lanoline paste containing either 0.5% TIBA or Q on the rate of petiole abscission in privet (*Ligustrum ovalifolium*) as compared with control

y-axis = the number of separated petioles, x-axis = time

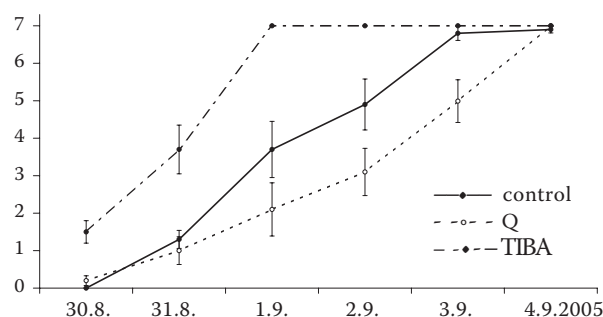


Figure 2. Effect of the lanoline paste containing either 0.5% TIBA or Q on the rate of apple petioles abscission as compared with control

y-axis = number of abscised petioles, x-axis = time

of cultivation, i.e. on 4 August 2005. During the decapitation one cotyledon was cut off and the remaining one was treated either with a lanolin paste containing 0.5% Q or TIBA; one third was used as control (either untreated or treated with an aqueous lanolin paste). The growth of buds situated in axillas of removed and remaining cotyledons was evaluated after 15 days (i.e. on 19 August). As shown in Figure 4, the application of TIBA reversed the correlation existing between the remaining cotyledon and its axillary bud. In the control the growth of axillar of remaining cotyledon was significantly stronger than the growth of axillar of remaining cotyledon treated with the TIBA paste. The application of Q on the remaining cotyledon showed only a slight inhibiting effect on the growth of its axillar, which means that it is not possible to expect a similar reverse effect of Q application as in the case of TIBA application.

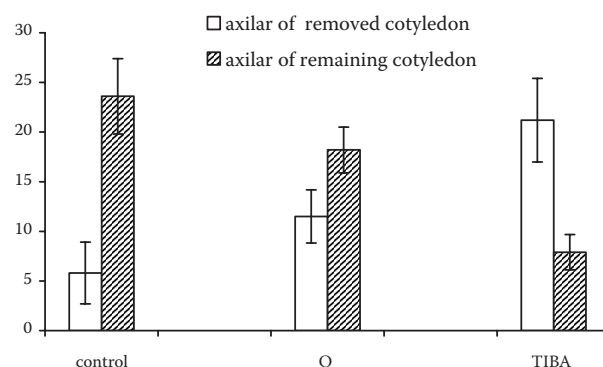


Figure 4. Growth of axillary buds on decapitated flax seedlings deprived of one cotyledon after the treatment of remaining cotyledon with the lanoline paste containing either 0.5% TIBA or Q as compared with control

y-axis = length of axillars, x-axis = application

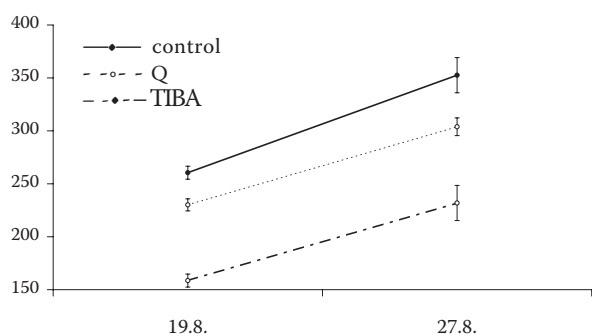


Figure 5. Growth of epicotyls of pea seedlings as influenced by the lanolin paste containing either 0.5% TIBA or Q as compared with control

y-axis = length of epicotyls, x-axis = time

GROWTH OF EPICOTYLS ON PEA SEEDLING

Pea seeds were sown on 5 August 2005. After one week of cultivation in darkness (i.e. on 13 August), seedlings 50-mm-long were treated in the apical part of epicotyls with the lanolin paste containing either 0.5% TIBA or Q. The remaining third of seedlings was used as control. Figure 5 shows that both applied substances inhibited the elongation growth of epicotyls but the effect of TIBA was considerably stronger than that of Q.

COMPARISON OF EFFECTS OF Q, IAA AND TIBA ON ETHYLENE PRODUCTION

As shown in Figure 6, IAA promoted the ethylene production most from the very beginning of the measurements. As compared with IAA, the stimulating effect of Q was delayed at first but thereafter it equalled that of IAA. The effect of TIBA on the ethylene production was less marked and it was equal to those of IAA and Q only as late as in the last stage of this experiment.

DISCUSSION

The inhibiting effect of IAA on the abscission of leaf petioles in apple tree, as described earlier by Šebánek (1978), was confirmed in this study. TIBA is an inhibitor of polar transport of auxin (Niedergang-Kamien and Skoog 1956, Nam-Myung Hee et al. 1999) and this is in accordance with its promoting effect on abscission of petioles

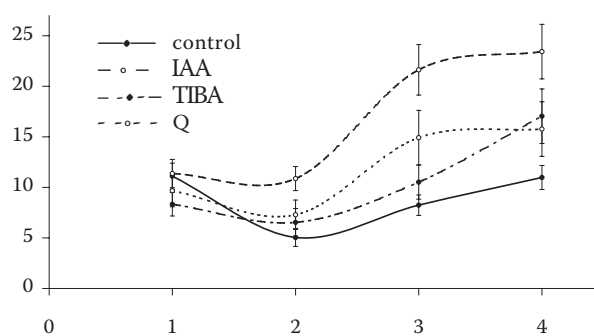


Figure 6. Effects of the lanoline paste containing either 0.5% TIBA, Q or IAA on ethylene production in pea seedlings as compared with control

y-axis = ethylene concentration (nl/l), x-axis = sampling time

in apple trees observed in this study. However, in privet (*Ligustrum ovalifolium*) petioles TIBA showed a slight inhibiting effect on the abscission of these petioles. Similar results were obtained also in our previous study (Šebánek et al. 1999) with privet (*Ligustrum vulgare*), in which TIBA in 0.1% concentration showed an inhibiting effect on abscission but in 0.5% concentration no effect was observed. However, it is very interesting that Q, which is considered to be an inhibitor of polar auxin transport, showed a very marked inhibiting effect on the abscission of petioles. This inhibiting effect was similar to that of IAA in the experiments with privet and apple trees. On decapitated and one-cotyledon-deprived flax seedling the growth of the remaining cotyledon is usually more intensive; this is associated with an auxin-like effect of this cotyledon (Dostál 1967). For that reason TIBA reversed this correlation after its application on the remaining cotyledon, which was demonstrated in this study. Quercetin did not show an inhibiting effect on auxin transport in this case because after its application on the remaining cotyledon the growth of its axillary bud was inhibited only insignificantly while that of TIBA was very pronounced. As far as the growth of epicotyls of pea seedlings was concerned, TIBA showed marked inhibiting effects and this corresponded with the results published by Ross (1998). The effect of Q was only slightly inhibiting, which indicates that, in spite of the fact that both Q and TIBA are considered to be the inhibitors of auxin transport, they do not show any similar morphogenetic and/or growth inhibiting effects; in case of petiole abscission of apple trees these effect are even opposite. However, it is necessary

to remember that both compounds show a number of other effects that can influence studied processes in an indirect manner. So, for example, TIBA inhibits the uptake of ions (Jacobson and Jacobson 1980) and Q inhibits Ca^{2+} -dependent hydrolysis of ATP (Shoshan et al. 1980), i.e. processes that can significantly influence physiological effects of auxin.

In our experiments IAA increased the production of ethylene and this observation is in compliance with previous data (e.g. Macháčková 1997). However, there were no contrary effects of IAA and inhibitors of its transport (Q and TIBA) on ethylene production; in the final phase of this experiment the effects of Q and IAA even showed to be similar.

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