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Reversal of Auxin Induced Inhibition by Ethrel

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Dedicated to the commemoration of Professor PhDr. Dr.h.c. Rudolf Dostál, DrSc.

Abstract. The interaction between exogenous 2-chloroethylphosphonic acid (Ethrel, CEPA) and auxin (both native and synthetic — IAA) was studied on pea and bean seedlings, potato tubers, and processed flax plants. After the addition of ethrel the inhibiting effect of IAA was decreased in all objects and it was found that the concentration of the growth of the regulators played an important role. The growth response of a part of flax hypocotyl, as induced by exogenous auxin produced in the cotyledon, was reversed by ethrel, too. The application of ethrel on the epicotyl apex in beans resulted in the lost of apical dominance of epicotyl and in the growth of lateral buds together with the epicotyl. When stimulating the growth, ethrel reverses the inhibitions through the decrease in the auxin content (from an inhibiting, supraoptimum level to an optimum one which already stimulates growth). In objects with a low content of endogenous auxin the ethrel induced the decrease in the auxin content and shows an inhibiting effect on growth.

The results of studies on the effect of the morphoregulator ethrel on plants gave the information on its relation to the spectrum of native growth regulators and, in the studies on the reaction of ethrel with exogenous regulators, on its interaction with these substances. Sankhla and Shukla (1970) and Hradilík (1972a) studied the relationship of ethrel to exogenous gibberellin and found out using various objects that ethrel decreased the stimulative effect of gibberellin on the elongation growth of plant organs. Hradilík (1974) in an experiment with radish cotyledons demonstrated that ethrel also decreased the stimulative effect of 6-benzylaminopurine on the production of fresh matter of radish cotyledons induced by this cytokinin. Sankhla and Sankhla (1972) observed a reversal of the germination inhibition in lettuce seeds (*Lactuca sativa* L.) by ethrel. Hradilík (1972, 1973) described the antagonism between endogenous auxin produced in the epicotyl apex of germinating seedlings of pea (*Pisum sativum* L.) and exogenous ethrel which, in many cases, caused a temporary reversal of the apical dominance and induced the growth of axillars on plants with an intact epicotyl.

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The stimulating effect of ethrel on the growth of lateral, correlatively-inhibited buds and the ability to break seed dormancy (IYER *et al.* 1970; OBHLÍDALOVÁ and HRADILÍK 1973) suggest a marked ability of ethrel, and thus "de facto" of ethylene released by this substance (EDGERTON and BLANPIED 1968; PRATT and GOESCHL 1969; YANG 1969), to interfere with the endogenous regulation system.

This study is an attempt to explain interactions between both endogenous and exogenous auxin and ethrel in various botanical objects.

Material and Methods

Pea (*Pisum sativum* L. cv. Raman)

(a) Pea seeds were imbibed in an ethrel 68—250 solution of various concentrations (2-chloroethylphosphonic acid; CEPA), manufactured by Amchem Co. (Ambler, USA), auxin (indole-3--acetic acid, IAA), manufactured by Lachema Co. (Brno, Czechoslovakia) and in the mixtures of these substances.

Control seeds were imbibed in distilled water. After 12 h all the treatments were washed with water, placed into damp sawdust (radices directed downwards), and incubated in a dark thermostat at 26 °C for 96 h. Then the germination capacity was determined. Each variant corresponded with 100 to 150 seeds and three replications were used.

(b) After 12 h of soaking in water the pea seeds were placed into damp sawdust and incubated in a dark thermostat at 20 °C for four days. Thereafter the germinating plants were washed with water, deprived of testae, decapitated, and established as a water culture without mineral nutrition. Three days later auxin paste was applied onto the epicotyl stump (0.5 per cent IAA — lanoline + water 1 : 1), in one half of the plants the ethrel paste (0.5 per cent CEPA — lanoline + water 1 : 1) was applied on one cotyledon. The plants were kept in darkness at 20 °C (relative humidity 75 ± 5 per cent) and the evaluation was carried out after five days. Each treatment consisted of 50 plants. The experiment was replicated four times.

(c) After 12 h of soaking in water pea seeds were left to germinate in the sawdust and 30 mm long epicotyls were treated according to WENT (cf. MITCHELL and LIVINGSTON 1968).

Bean (*Phaseolus coccineus* L.)

Bean seeds were germinated similarly as those of pea; the only difference was that all the seeds were left in the sawdust till the end of the experiment. Ethrel was applied onto epicotyls of etiolated, two-days-old plants in the form of lanoline paste.

Potato (*Solanum tuberosum* L.) cv. Jiskra

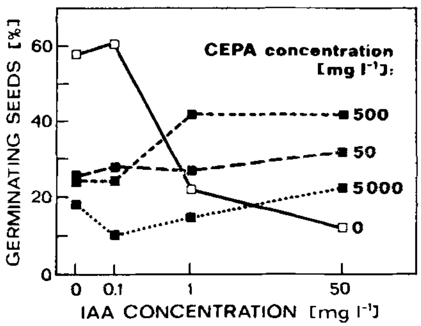
Potato tubers of a uniform size (altogether 5 to 6 kg) were soaked for 5 min in ethrel, auxin, and mixed solutions on February 13, 1973 (*i.e.* in the period when endogenous dormancy is finishing). The treated material was placed into aluminium boxes and incubated in a dark thermostatized room (26 °C; relative humidity 75 ± 5 per cent). The growing buds were counted after 14 days.

Flax (*Linum usitatissimum* L.) cv. Reina

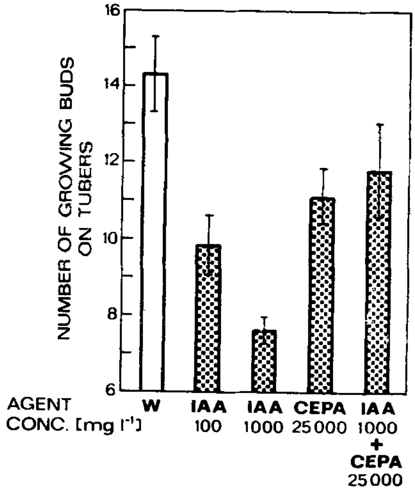
In this case a method described by DOSTÁL (1968) was used:

Segments (25 mm below the cotyledons deprived of apexes and in some cases also of one cotyledon) were obtained from 10-day-old plants grown under fluorescent lamps, placed into polystyrene boxes, and transferred in water under fluorescent lamps (3 000 lx).

The growth regulators were applied onto cotyledons in the form of lanoline paste or agar blocks; in controls water lanoline paste and agar blocks (1 per cent agar, 3 × 3 × 3 mm) were used. Since the application of regulators the material had been observed continuously and evaluation were made after 24 and 48 h.



▲ Fig. 1 The effect of auxin (IAA), ethrel (CEPA) and their mixtures on germination of pea seeds.



▶ Fig. 2. The effect of auxin, ethrel (CEPA) and their mixtures on growth of potato buds.

Results and Discussion

In pea the exogenous auxin (IAA) induced inhibition of growth processes may be reversed by exogenously applied gibberellin (ŠEBÁNEK 1972), synthetic cytokinin (SACHS and THIMANN 1964, 1967), native cytokinin (SANKHLA and SANKHLA 1968), and also ethrel (SANKHLA and SANKHLA 1972). The results obtained in this study with various materials, differing considerably from the genetical point of view, suggest that ethrel is able to reverse the auxin (IAA) induced growth inhibition.

IYER *et al.* 1970; OBHLÍDALOVÁ and HRADILÍK 1973 observed that ethrel reversed also the inhibition of seed germination induced by native substances during dormancy.

Both ethrel and higher doses of IAA inhibited the germination of pea (Fig. 1). However, the auxin (IAA) induced the inhibition of germination of pea seeds and was markedly reversed by the addition of ethrel (Fig. 1 — 50 mg IAA l^{-1}). Partially quantitative relationships may be observed, too. The 5 000 mg l^{-1} concentration of ethrel was too high and for that reason it showed an inhibiting effect in mixtures with auxin (IAA; 0.1 and 1.0 mg l^{-1}) as the inhibiting effect of ethrel prevailed.

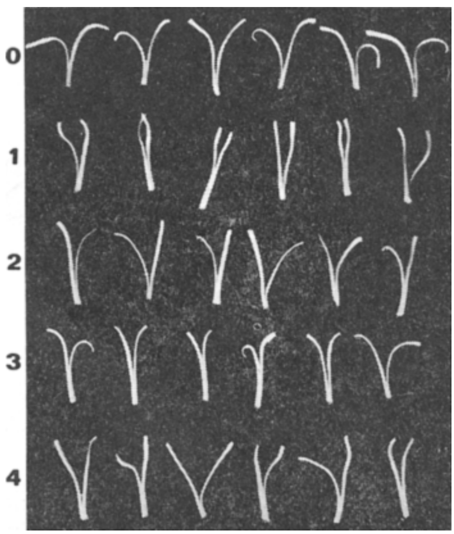


Fig. 3. The decreased effect of auxin on the curvature of pea epicotyls by ethrel. 0: control; 1: 0.1 mg l^{-1} IAA; 2: 10 mg l^{-1} CEPA; 3: 0.1 mg l^{-1} IAA + 10 mg l^{-1} CEPA; 4: 1 mg IAA + 10 mg l^{-1} CEPA.

The effect of ethrel was manifested at a concentration of 50 mg l^{-1} IAA in the form of a decreased auxin inhibition. The most marked reversal of this auxin induced inhibition occurred after the application of ethrel solution in the concentration of 500 mg l^{-1} (Fig. 1).

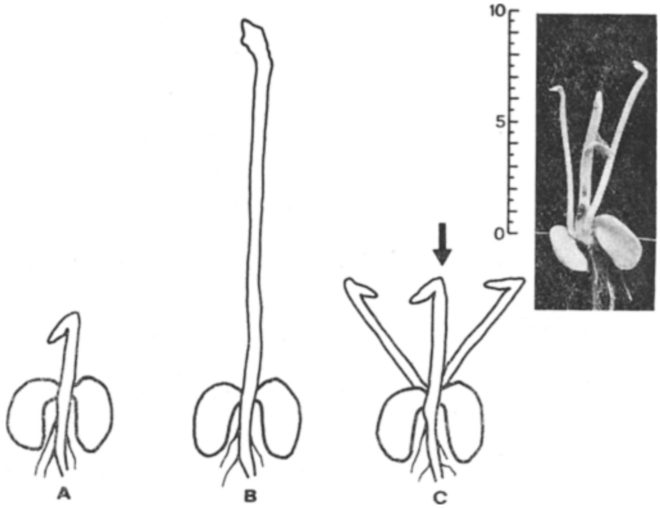


Fig. 4. A Scheme of the effect of ethrel (CEPA 5 000 mg l^{-1}) on growth of bean seedlings. *A* — bean seedlings at the time of application; *B* — plants treated with a water paste; *C* — plants treated with a CEPA paste (20 per cent); photograph — idem (scale in cm).

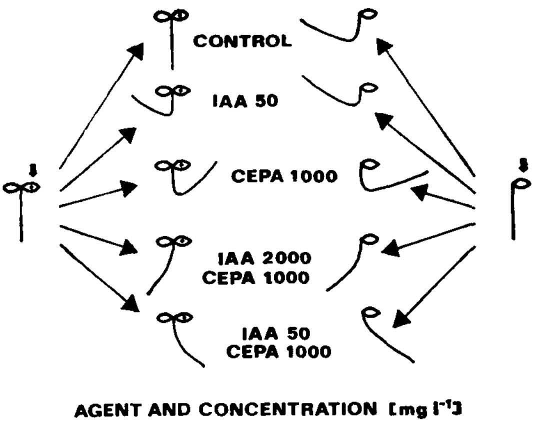


Fig. 5. The schematic presentation of results of an experiment with decapitated and root-deprived flax plants (left) and with the same plants deprived also on cotyledon (right). Regulators were applied onto the cotyledon.

In the experiment with potato tubers [see Material and Methods sub. (b)] ethrel markedly reversed the inhibiting effect of auxin on the growth of buds in that variety which was not dormant (Fig. 2); however, ethrel alone decreased the number of growing buds in this variety and in this period (February 13), *i.e.* it showed an inhibiting effect. When applied on deeply dormant tubers of the same variety (*i.e.* approximately two months earlier) ethrel increased the number of growing buds and, therefore, showed a stimulating effect (HRADILÍK 1973a).

Similarly, in the experiment with splitted pea epicotyls [see Material and Methods sub. (c)] a quantitative relationship may be observed in the reaction in which ethrel reversed, in dependence on its concentration, partly or completely the effect of IAA on the curvature of splitted pea epicotyls (Fig. 3).

In our earlier studies (HRADILÍK 1972, 1973) the stimulating effect of ethrel on cotylar growth of intact pea plants was described. In this study the aforementioned effect was demonstrated in beans, too (Fig. 4).

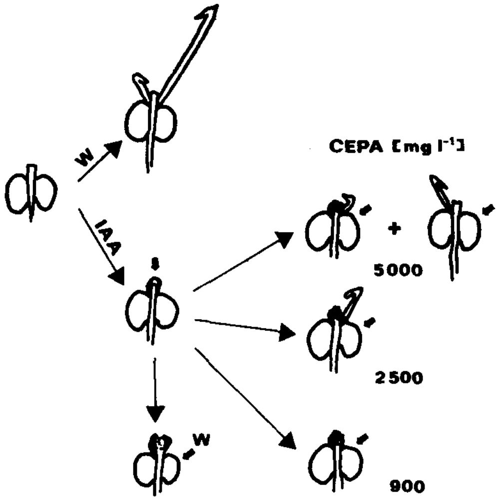


Fig. 6. The reversal of auxin (0.5 per cent IAA) inhibited growth of pea cotylars by CEPA. W — lanoline paste without regulators.

This disturbance of the apical dominance probably resulted from the increased content of native auxin after the ethrel treatment.

The curvature of the hypocotyl plant stump provided information consistent with data obtained in experiments with aforementioned objects. Auxin, both native and synthetic (IAA) applied exogenously on cotyledons, induced the growth of the hypocotyl stump in the direction of the treated cotyledon (DOSTÁL 1968). This curvature was reduced or reversed in dependence on the ethrel concentration. Ethrel alone, applied onto the cotyledon, induced the curvature of the hypocotyl below this treated cotyledon. In decapitated flax plants, with one removed cotyledon, the hypocotyl stump bended away from the cotyledon. This curvature (induced by substances produced in the remaining cotyledon) was further amplified by the exogenous IAA (DOSTÁL 1968); however, through the effect of ethrel on the cotyledon a reversal of the bending occurred and the hypocotyl stump bended below the cotyledon. (Fig. 5).

The presumption that the biosynthesis of gibberellins and cytokinins could be induced by ethrel controverts results describing the antagonistic relationship between ethrel on the one hand and gibberellin and cytokinin on the other (SANKHLA and SHUKLA 1970; HRADILÍK 1972a, HRADILÍK 1974). To explain the causes of lateral bud growth in beans it is possible to start from the hypothesis of a "direct inhibition" according to which the growth of these lateral buds is inhibited by a high level of native auxin transported from the epicotyl apex. This coincides with the fact that also synthetic auxin (IAA) applied on the epicotyl stump of a decapitated pea plant e.g. in the form of a 0.5 per cen paste inhibits completely the growth of cotylars (THIMANN and SKOOG 1933, 1934). On the other hand a relief of lateral buds from the correlative auxin inhibition occurs even on an intact plant after the application of ethrel (HRADILÍK 1972, 1973; see Fig. 4).

Ethrel applied in the form of a 0.25 per cent paste on one of both cotyledons broke in 20 per cent of cases the auxin induced inhibition resulting from the application of a 0.5 per cent IAA paste on the epicotyl stump of decapitated etiolated pea plants, too (Fig. 6).

In this experiment, as well as in all the above discussed cases, the concentration of ethrel is of a great importance. A high concentration of ethrel (0.5 per cent) stimulated the growth of cotylar in the axilla of treated cotyledon in 5 per cent of plants; in 10 per cent of plants this concentration stimulated the growth of cotylar in the axilla of untreated cotyledon, probably as a result of the induced action of ethrel on this cotylar.

Ethrel applied in the form of a 0.25 per cent paste on cotyledons of decapitated, inhibited (by 0.5 per cent IAA) pea plants reversed this auxin induced inhibition of cotylar growth in 20 per cent of treated plants while ethrel, applied on one cotyledon of decapitated pea plants, induced the growth of cotylars situated in axillas of treated cotyledon in all cases (HRADILÍK 1972, 1973). The lowest of all the concentrations used, a 0.09 per cent paste, was not able to reverse the auxin induced inhibition and to stimulate the cotylar growth (Fig. 6).

Conclusions

Considering results presented in this paper and comparing with data of aforementioned authors, it is possible to draw the following conclusions about the relationship between auxin (IAA) and ethrel:

When stimulating the growth ethrel reverses the inhibitions through the decrease in the auxin content (from an inhibiting supraoptimum level to an optimum one which already stimulates the growth). In objects with a low content of endogenous auxin the ethrel induced decrease in the auxin content shows an inhibiting effect on the growth, *e.g.* after the application of ethrel on cotyledons of decapitated pea plants where this substance inhibits the growth of cotylars in axillas of treated cotyledons (HRADILÍK 1972, 1973).

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Interakce mezi exogenně aplikovanou kyselinou 2-chlorethylfosfonovou (Ethrel, CEPA) a auxinem ať již nativním, nebo syntetickým (IAA) byla studována na klíčících rostlinách hrachu a fazolu, na hlízách bramboru a částech rostlin lnu. Inhibiční účinek IAA byl snižován u všech objektů přídavkem ethrelu, přičemž významnou roli zde hraje koncentrace používaných růstových regulátorů. Také růstová reakce části hypokotylu lnu indukovaná endogenním auxinem produkovaným dělohou byla ethrelem zrušena. Aplikace ethrelu na vrcholek epikotylu fazolu vedla ke ztrátě apikální dominance epikotylu a růstu postranních pupenů za současné existence epikotylu.

Tam, kde ethrel vykazuje stimulační účinek na růst, ruší inhibice tím, že snižuje obsah auxinu z inhibiční supraoptimální hladiny na hladinu optimální, která již podněcuje růst. U objektů, které obsahují málo endogenního auxinu působí snížení obsahu nativního auxinu na růst inhibičně.