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Interaction between auxin, kinetin and citric acid in relation to apical dominance

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INTRODUCTION

Until recently our knowlegde of the mechanism of apical dominance amounted to five basic observations; 1° that lateral buds are inhibited by the terminal bud and can be released from that inhibition by the removal of the terminal, 2° that β-indoleacetic acid (IAA) can substitute for the terminal bud in its inhibitory influence on the lateral, 3° that laterals have little auxin and much inhibitors comparative to the terminal, 4° that removal of the terminal results in an increase of auxin in the lateral and 5° that the addition of weak IAA can stimulate the growth of the lateral (Pilet 1961). More recently (Wickson and Thimann 1958, von Maltzahn 1959) it was shown that the inhibitory effect of IAA on laterals can be reversed by kinetin (Ki).

The observations are rather puzzling since it is hard to explain why IAA which normally has a growth promoting effect should inhibit. Also the natural increase of auxin in a released lateral seems to be in contradiction with the observation that auxin prevents its release.

Recently several attempts were made at explaining the mechanism in nutritional terms. Kuse (1961) has shown that in *Ipomoea batatas* Lam. when the axis is decapitated old leaf stimulated axillary bud growth and young leaf inhibited it. The inhibitive effect of the young leaf could be replaced by auxins applied to the petiole. The further away from the axis the application was made the greater was the inhibition. Glucose reversed the inhibitory effect of the leaf. There exists a negative correlation between axillary bud growth and the growth of other parts of the leaf, suggesting a competition for nutrients. This would amount to indirect inhibition. However the author points out that there is direct inhibition in the case of control of lateral buds when the auxin is known to reach the base of the lateral bud.

Jacobs and Bullwinkel (1953) working on *Coleus* have shown that when side buds and side shoots are excised the main shoot grows more, and in this case IAA in place of the laterals has no inhibitory effect on the main shoot. This again would suggest that the greater

growth of the main stem is the result of increased nutritional supply, which otherwise would have been distributed also among the laterals.

A special interpretation of the nutritional condition was provided by the observation that points of a leaf treated with Ki will accumulate amino acids, even without protein synthesis. In young leaves the effect is less, presumably due to the presence of natural kinins keeping the amino acids (Mothes and Engelbrecht 1961). This was found to be also true for IAA and it was shown that Ki will accumulate both natural and applied auxin (Conrad 1961). Possibly the accumulatory power of Ki with respect to amino acids is through auxins. The movement of ^{14}C labelled glucose to IAA treated parts of the shoot was demonstrated for several plants (Booth *et al.* 1962). It could be that the role of the applied auxin in place of the apical bud is to accumulate metabolites which otherwise could stimulate the release of laterals. However the similar accumulatory power of Ki fails to explain why Ki reverses the IAA inhibition of laterals.

In peas, where Ki failed to reverse the IAA effect (Champagnat *et al.* 1960), on provision of metabolites in the form of organic acids, the Ki and the metabolites were synergistic in stimulating the growth of the laterals inhibited by IAA (Champagnat and Dassouval 1960). This would again suggest that an explanation of apical dominance may be found in the competition for nutrients.

However the nutritional explanations fail to account for the observation that ^{14}C labelled IAA actually enters the laterals and its content in the laterals is inversly related to the growth of the lateral (Wickson and Thimann 1960).

For completeness it should be also mentioned that what Jacobs *et al.* (1959) believe to be an auxin concentration exactly substituting for that supplied by the removed apex fails to inhibit the laterals.

In the discussion of the problem little use has so far been made of the information about the state of the lateral bud before the removal of the terminal. It can be assumed, that since the lateral is not expected to produce a very long shoot it contains an axis with a restricted number of cells. It seems reasonable to suppose that the removal of the terminal bud will divert the nutrient supply to the laterals, and this alone may constitute the stimulus for the release of the lateral. But the growth of the lateral will of necessity comprise cell division as well as cell elongation. These two processes are to some extent competitive as has been shown in studies on artichoke tuber tissue (Adamson 1962) where rate of cell elongation was shown to be inversly proportional to the rate of cell division. The author suggested that when cell expansion gets too fast, division cannot take place, this being the normal state of events below an apical meristem.

In the case of the released lateral it is possible that a certain natural

balance between cell division and cell elongation takes place resulting in optimal growth. IAA is the classical hormone responsible for stimulation of cell elongation. When it is applied in place of the terminal it may result in the elongation of existing cells in the lateral bud at a rate depriving them of meristematic activity, which in effect amounts to inhibition of the undeveloped bud. On the other hand Ki is a cell division factor and as such may counteract the inhibitory effect of IAA--induced rate of cell elongation. The requirement for metabolites is understandable if both cell division and cell elongation are to proceed at a fast rate.

To test this hypothesis it was decided to try the effect of IAA, Ki an a metabolite on the growth of laterals in a plant which has cell division and cell elongation separated in time. This is believed to be the case in pine species, which do have in the spring a period of stem elongation consisiting of the elongation of organs already constituted in the bud, followed by a period of cell division and bud differentiation.

MATERIALS AND METHOD

For the study were chosen five 28-year old yellow pine trees (*Pinus ponderosa* Dougl.) growing in the Kórnik Arboretum.

The experiment was performed on the 27th. of April 1963 when the buds were just beginning to elongate. Shoot apices were chosen which had a well developed terminal bud and one definite lateral bud on the terminal cluster. Eleven different treatments were made.

1. A control with the buds left intact and no chemical applications made.
2. The lateral buds were removed by pinching and in their place pure lanoline paste was applied.
3. The lateral buds were removed and in their place lanoline paste containing 1 mg of IAA per 1 ml was applied.
4. The terminal bud was removed and in its place pure lanoline was applied.
5. The terminal bud was removed and in its place lanoline paste containing 1 mg of IAA per 1 ml was applied.

The following treatments differ from no. 5 in the lanoline preparations which were as follows.

6. 1 mg Ki per 1 ml of lanoline.
7. 0.1 gm of citric acid per 1 ml of lanoline.
8. 1 mg IAA + 1 mg Ki per 1 ml of lanoline.
9. 1 mg IAA + 0.1 gm citric acid per 1 ml of lanoline.
10. 1 mg Ki + 0.1 gm citric acid per 1 ml of lanoline.
11. 1 mg IAA + 1 mg Ki + 0.1 gm citric acid per 1 ml of lanoline.

All eleven treatments were made on each of the five experimental trees. The experiment was treated as a random block design. Citric acid was chosen as a representative metabolite which was found to synergise with Ki in the reversal of the inhibitive effect of IAA on peas (C h a m p a g n a t and D a s s o u v a l 1960).

The extension growth of the remaining buds and shoots that developed from them was measured periodically. If more than one lateral existed, always the largest one was considered. The experimental differences were observable three days after the experiment was started, and the basic relations did not change later, however the statistical significance of the results increased with time and so only the final results as measured June 1st. are reported. Also the number of new lateral buds that developed on the treated shoot apices was scored on June 10th.

RESULTS

Table 1. gives the measurements of the shoot length in relation to the eleven treatments for the 5 replicates. It can be seen that there are considerable differences in growth between the replicates, each replicate tree having its own rate of growth. The differences due to treatments are also substancial. Fig. 1 compares the effect of debudding with the control. It can be seen that the removal of either terminal or lateral buds in April does not affect the growth of remaining buds during May.

Table 1

Shoot lengths in cm. 35 days after the experimental treatments were given

	Treatment	Replicates 1	2	3	4	5	Average	Total No. of new buds
1	Control { Terminal	6.0	10.4	12.0	10.0	8.0	9.3	0
	Control { Lateral	1.0	4.2	4.9	3.2	6.5	4.0	
2	Terminal blank	6.0	11.0	10.0	12.6	4.8	8.9	0
3	Terminal+IAA	5.5	7.8	14.4	9.5	8.1	9.1	0
4	Lateral blank	0.9	1.9	11.2	2.4	6.1	4.5	10
5	Lateral+IAA	1.0	8.7	9.7	7.8	2.1	5.9	1
6	Lateral+Ki	1.3	2.3	6.8	2.0	1.7	2.8	10
7	Lateral+C.A.*	1.2	5.6	7.2	5.0	7.6	5.3	7
8	Lateral+IAA+Ki	1.4	5.4	7.1	0.5	3.1	3.5	1
9	Lateral+IAA+C.A.	1.8	4.0	8.5	3.0	1.1	3.7	5
10	Lateral+Ki+C.A.	3.8	6.3	5.1	3.3	1.5	4.0	7
11	Lateral+IAA+Ki+C.A.	1.4	3.1	1.5	1.0	0.3	1.5	4

* C.A. = citric acid

Also a comparison of treatments 2 and 3 (Table 1) indicates that application of IAA in place of the removed lateral buds has no effect on the size of the terminal shoot.

In Fig. 2 a comparison is made of the growth of laterals after the replacement of a removed terminal bud by different lanoline preparations. D is the minimum difference that can be considered as statistically

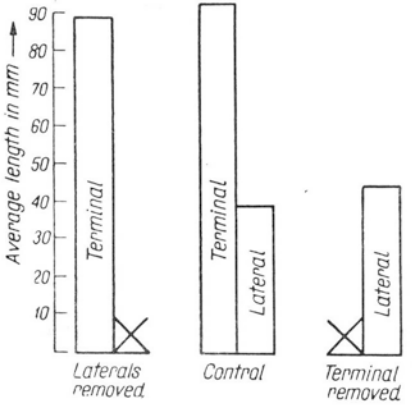

Fig. 1. Effect of bud removal on the growth of remaining buds.

Wpływ usuwania pączków na wzrost pączków pozostałych

significant. It can be seen that both IAA and citric acid somewhat increase the growth of laterals, whereas Ki lowers it. When both Ki and IAA or Ki and citric acid are given, the growth of the laterals is intermediate between that shown by the shoots given a single chemical. This suggests that the effects of Ki and the other two substances are additive.

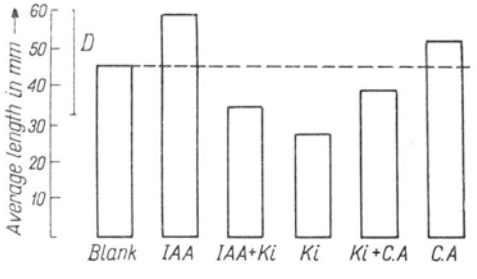

Fig. 2. Growth of a lateral shoot after the replacement of the terminal by hormonal preparations or citric acid (C.A.). D is the minimum difference that can be considered as statistically significant.

Wzrost pędu bocznego po zastąpieniu pędu głównego preparatem hormonalnym lub kwasem cytrynowym (C.A.). D — minimalna różnica statystycznie istotna

Figure 3 demonstrates the rather unexpected interaction between IAA and citric acid. When given singly both IAA and citric acid on the average increased the growth of the laterals compared with controls. However when given together the growth is inhibited to the extent that

it becomes lower than that for the controls. Similarily when to the IAA + Ki preparation citric acid is added the growth of the laterals is lowered.

The results of treatments 4 to 11 were subjected to an analysis of variance. Table 2 gives the relevant figures of the analysis. Certain

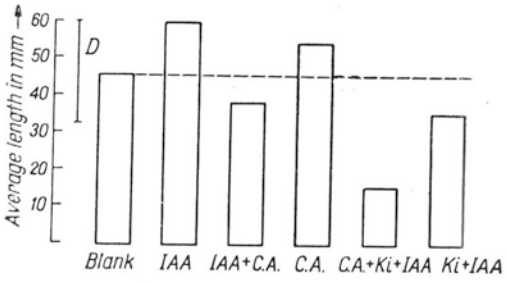

Fig. 3. Antagonism between IAA and citric acid (C.A.). *D* is the minimum difference that can be considered as statistically significant.

Antagonizm między IAA a kwasem cytrynowym (C.A.). *D* — minimalna różnica statystycznie istotna

significant differences are demonstrated. Firstly Ki has a highly significant (1% level) depressing effect on the growth of lateral buds. This confirms the observations demonstrated on Fig. 2.

Secondly the interaction between IAA and citric acid is significant (5% level). This implies that the values seen in the IAA — citric acid interaction table are significantly different from each other.

Table 3 is the interaction table in question. It can be seen from the totals that the overall effects of IAA and citric acid are obscured by the interaction between them. In reality both IAA an citric acid have

Table 2

Analysis of variance table for the treatments 4 to 11

	d.f.	S.S.	M.S.	f
Total	39	331.59		
Treatments	7	67.78	9.68	2.25
IAA	1	2.86	2.86	—
Ki	1	35.91	35.91	8.33**
C.A.	1	3.08	3.08	—
IAA × Ki	1	1.56	1.56	—
IAA × C.A.	1	24.18	24.18	5.61*
Ki × C.A.	1	0.16	0.16	—
IAA × Ki × C.A.	1	0.03	0.03	—
Trees	4	143.04	35.76	8.29**
Residual	28	120.77	4.31	

$f_{1,28}$ = 4.20 at 5% level and 7.64 at 1% level.

Table 3

Interaction table for IAA and citric acid. The values are totals as used in the analysis of variance

	Citric acid	Blank	Total
IAA	25.7	46.8	72.5
Blank	46.6	36.6	83.2
Total	72.3	83.4	155.7

some promoting effect on the growth of the laterals compared with the blank, but when they are given together they antagonise each other resulting in a growth depression.

The promotive effect of IAA and of citric acid on the growth of the laterals cannot be considered as proven, particularily since there is considerable variation among the replicates, however it is strongly indicated. A comparison of treatments 4 and 5 and treatments 6 and 8 (Table 1) indicates that in 7 cases out of 10 the effect of IAA without citric acid was to promote the growth of the laterals. Similarily comparing treatments 4 and 7, and treatments 6 and 10 indicates also that in 7 cases out of 10 the effect of citric acid without IAA was promotive on the growth of the laterals.

The highly significant difference between the replicate trees indicates that they have different growth rates.

From Table 1 it can also be seen that the removal of the terminal bud (treatment 4) results in the development of several new lateral buds. This development is inhibited by the lanoline preparations containing IAA (treatments 5, 8, 9, and 11).

DISCUSSION

The results can be summarized by saying that at the time of cell elongation the removal of a terminal bud results in 1° the production of extra buds, which are inhibited by IAA and 2° in the normal growth of lateral buds which are somewhat promoted by IAA and citric acid but inhibited by Ki. This is contrary to most of the observations reported in the literature. However, as has been suggested in the intruduction, the breaking away of laterals is predominantly the result of increased cell division, and so the growth during the period of cell elongation need not respond in the same way. It is suggested that IAA stimulates cell elongation and inhibits cell division, whereas Ki has the opposite effect.

Under normal conditions, when the terminal bud is in its place, competition for nutrients could maintain the lateral in an inhibited state.

When the terminal is removed, the nutrients are diverted to the lateral and whence the break away. Also the wounding involved in the removal of the terminal bud may result in the production of traumatins which stimulate meristematic activity on the wounded surface. Both the extra nutrient supply and the possible production of traumatins will result in increased cell division essential for the break way of the lateral. Increased meristematic activity will in turn increase auxin production which is known to increase in the lateral during its break away. If such an interpretation is true, under conditions where there is no cell division possible, there should be no break away. In the present investigation the removal of the terminal alone did not affect the growth of the lateral. This interpretation also conforms with the observation of Jacobs (*et al.* 1959) that the inhibition of the lateral is not obtained through an auxin supplied by the terminal.

Under conditions where cell division and cell elongation are simultaneous the application of IAA in place of the terminal is known to inhibit the growth of the lateral. It has been pointed out that while endogenous auxin travels only basipetally applied IAA shows some acropetal movement (Oserkowsky 1942). Thus it can be expected that applied IAA reaches the apical meristem of the lateral bud whereas normally the meristem only exports auxin that it produces. The abnormal supply of IAA to the meristem may stimulate the extension growth of the meristematic cells and thereby deprive them of meristematic activity (Adamson 1962). This would explain why the degree of inhibition of the lateral bud was proportional to the content of applied ^{14}C labelled IAA that reached the lateral (Wickson and Thimann 1960). The reversal of the IAA effect by Ki fits in well with this interpretation, since Ki will stimulate cell division that IAA has inhibited. On the other hand when no cell division is possible applied IAA should result in increased cell elongation only and Ki should direct cells towards meristematic activity without effectively achieving it and as a result reduce cell extension. The results of the present studies conform with this interpretation, since IAA has somewhat stimulated growth of the lateral and inhibited the production of new lateral buds, whereas Ki inhibited the growth of the laterals.

The slight stimulatory effect of citric acid indicates that the nutrient supply to the lateral, even when the terminal is removed, is less than optimal.

No satisfactory explanation can be given for the antagonism between IAA and citric acid.

The investigation is being continued on the same trees during the summer period when only cell division takes place in the buds and no further extension growth is expected until next year.

SUMMARY AND CONCLUSIONS

So far the investigations on apical dominance were coducted on plants in which cell division and cell elongation act simultaneously during growth. In pine the two processes are separated in time. Just prior to the period of stem elongation, on five *Pinus ponderosa* Dougl. trees growing in the Kórnik Arboretum, terminal twigs were subjected to eleven different treatments aimed at establishing the influence of IAA, kinetin and citric acid applications on extension growth in the absence of activity in the apical meristem.

1. The removal of terminal or lateral buds has no significant effect on the extension growth of the remaining buds.
2. When the terminal bud is replaced by IAA or citric acid the growth of the laterals is slightly stimulated.
3. Kinetin has the effect of inhibiting the growth of the laterals.
4. IAA and kinetin, and citric acid and kinetin act additively.
5. IAA and citric acid are antagonistic.
6. IAA inhibits the initiation of new lateral buds.

It is suggested that the inhibitory effect of IAA on laterals that has so often been reported, is an inhibition of cell division which is essential for the break away of lateral buds and which cannot take place under conditions of rapid cell elongation.

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Interakcja auksyny, kinetyny i kwasu cytrynowego w stosunku do zjawiska dominacji wierzchołkowej

Streszczenie

Dotychczasowe badania nad dominacją wierzchołkową były prowadzone na roślinach, u których podział komórkowy oraz wydłużanie się komórek następują równocześnie podczas wzrostu. U sosen te dwa procesy są oddzielone w czasie. W chwili rozpoczynania wydłużania się pączków, na pięciu drzewach *Pinus ponderosa* Dougl. rosnących w Arboretum Kórnickim, pędy końcowe poddane były jedenastu różnym zabiegom, mającym na celu ustalenie wpływu auksyny, kinetyny i kwasu cytrynowego na wzrost pączków w okresie pozbawionym aktywności merystemu wierzchołkowego.

1. Usunięcie pączka głównego lub pączków bocznych nie ma istotnego wpływu na wzrost pączków pozostałych.
2. Gdy pączek szczytowy zastąpiony jest roztworem kwasu indoliloctowego (IAA) lub kwasem cytrynowym, wzrost pączków bocznych jest przeważnie nieco zwiększony.
3. Kinetyna redukuje wzrost pączków bocznych.
4. IAA i kinetyna oraz kwas cytrynowy i kinetyna działają kumulatywnie.
5. IAA i kwas cytrynowy działają antagonistycznie.
6. IAA ogranicza formowanie się nowych pączków bocznych.

Na podstawie tych rezultatów autor konkluduje, że ograniczanie wzrostu pączków bocznych przez IAA, tak często w literaturze notowane, następuje na skutek inhibicji podziału komórkowego, który konieczny jest dla odbicia pączków bocznych, a który nie może następować w warunkach szybkiego wydłużania się komórek.