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THE EFFECT OF CAFFEIC ACID ON THE EPINASTIC CURVATURE OF ISOLATED PETIOLES OF COLEUS SP. IN COMPARISON WITH THE EFFECT OF GIBBERELLIC ACID AND INDOLEACETIC ACID

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Abstract

The isolated petioles of *Coleus* show a distinct dependence on indoleacetic acid in the development of the epinastic curvature, in aqueous medium. Under the same experimental conditions gibberellic acid shows no effect or an inhibition only.
Caffeic acid tested in concentrations between 10^{-11} and 10^{-8} evoked varying responses. In winter-grown plants the curvature *vs.* concentration curve shows two small maxima, which are not evident in the response of summer-grown plants.
The interaction of indoleacetic acid and caffeic acid is tested and discussed in elation with the question of the endogenous auxin in *Coleus*.

Introduction

Working on the abscission of debladed petioles of *Coleus* rhenaltianus, Vendrig (1960) reported the extraction of a substance, capable of retarding the abscission-time. Although this substance has an Rf value that does not clearly differ from indoleacetic acid under the same chromatographic conditions (Figs. 12, 13 and 14 of the paper mentioned), it was claimed to be a different substance, as three colour-reactions tried on the substance were negative as to proving its identity with indoleacetic acid. This substance was termed "*Abscission Retarding Substance*".

In a later paper Vendrig (1961) reported the presence of indole-derivatives in chromatograms of extracts of *Coleus*. But apart from these substances, he noticed the presence of two spots showing fluorescence in ultra violet light. One of these spots was identified as trans-caffeic acid, the other as a related compound not further determined. The two spots showing fluorescence in ultra violet light were eluted and tested in the Avena coleoptile section straight growth test, and were reported to show growth substance activity. This was also the case for commercial caffeic acid.

As the epinastic curvature of petioles of *Coleus* plants of the same clone as that used by Vendrig shows a growth substance dependence (Soekarjo, 1961), the present author has tried to obtain information on the activity of caffeic acid on the epinastic curvature.

Material and methods

The clone of *Coleus* used was the same as that used in earlier experiments by the present author (Soekarjo, 1961), used before by Terpstra (1956) and Vendrig (1960 and 1961).

The species name has been omitted in the title of this paper, so as not to cause unnecessary confusion, induced by the appearance of a species name differing from that used in the above mentioned papers by Terpstra and Vendrig. The plant has, however, been re-identified as belonging to the polymorph species *Coleus scutellarioides* Benth. For the identification of the plant material the author is indebted to Mr. J. H. Kern of the "Rijksherbarium" at Leiden.

The method used is a slight modification of the procedure described by Bottelier (1954).

The plants were grown in the greenhouse under long-day conditions. The shoots were harvested and kept in the dark for 24 hours with their basal ends in water. The petioles were then cut off and 10 petioles were put in a vial containing 100 ml of a test solution.

These manipulations were done in green light (emission between $\lambda = 500$ nm and $\lambda = 580$ nm, with an intensity of 0.4 μ watt·cm^{-2} on the level of the working bench).

After a stay of 24 hours in the test solution, shadowgraphs were taken and the curvatures measured by means of a goniometer.

The values of the curvatures are given as the mean together with the standard error of the mean and in brackets the number of individual measurements:

$$\bar{x} \pm \sqrt{\frac{\Sigma(x-\bar{x})^2}{n(n-1)}}\ (n).$$

These values are rounded off to the nearest degree.

The light in the experimental treatment was obtained from two Philips TL 40 W/33 fluorescent light tubes, suspended 60 cm above the working bench.

Table 1

The epinastic response of petioles of the fourth leaf pair to caffeic acid, in light and in darkness

Plants grown in winter

concentration caffeic acid	degrees curvature	
	in light	in darkness
10^{-10}	28 ± 3 (11)	23 ± 4 (10)
10^{-9}	37 ± 7 (9)	26 ± 5 (10)
10^{-8}	44 ± 5 (10)	37 ± 6 (10)
10^{-7}	38 ± 5 (11)	32 ± 3 (11)
10^{-6}	35 ± 5 (10)	29 ± 3 (10)
10^{-5}	42 ± 8 (10)	35 ± 6 (11)
10^{-4}	48 ± 7 (9)	33 ± 5 (11)
10^{-3}	31 ± 7 (6)	29 ± 3 (6)

EXPERIMENTAL

Using plants that have been grown in winter, the results obtained when the effect of caffeic acid was tested, were of the type given in Table 1 and Fig. 1. Two small maxima in the curvature *VS*. concentration curve can be seen, in darkness as well as in light.

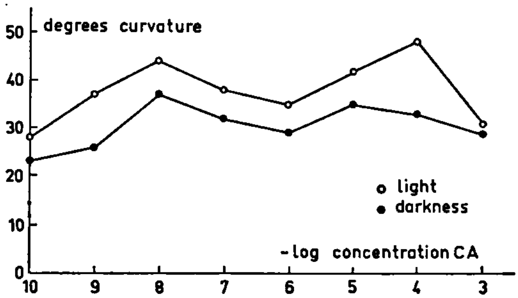

Fig. 1. The epinastic response of petioles of the fourth leaf pair to caffeic acid, in light and darkness.
Plants grown in winter.

Light is promoting the curvature at any given concentration of caffeic acid, in these plants. Experiments in summer did not give the same clear picture. Summer-grown plants do not show a clear light-dependence, the "summer" experiments were therefore done in darkness only. Table 2 and Fig. 2 show the results of three experiments of this type, in darkness only.

In all the experiments, there is but a small effect of caffeic acid on the curvature. In the youngest petioles used here (Fig. 2, A: the 4th leaf pair), there is hardly any promotion of the curvature at low concentrations of caffeic acid, even inhibition may occur. In the older petioles, however, there is a tendency of a promotion of the epinastic curvature by low concentrations of caffeic acid.

In higher concentrations of caffeic acid (above 10^{-7}) there is a small promotion in nearly all the cases.

Although there may be a certain effect of caffeic acid on the epinastic curvature, the magnitude of the curvature caused by caffeic acid is small as compared to the curvature caused by indoleacetic acid.

In Tables 3 and 4 and Fig. 3 the effect of indoleacetic acid and gibberellic acid (GA_3) on the epinastic curvature, in darkness as well as in light, is given as a comparison to the effect of caffeic acid.

The effect of gibberellic acid is clear: there is no effect or a small inhibition at the concentrations tested.

The curvature as a response towards indoleacetic acid is, however, very strong and the magnitude of the curvatures reach twice the values obtained as a response to caffeic acid.

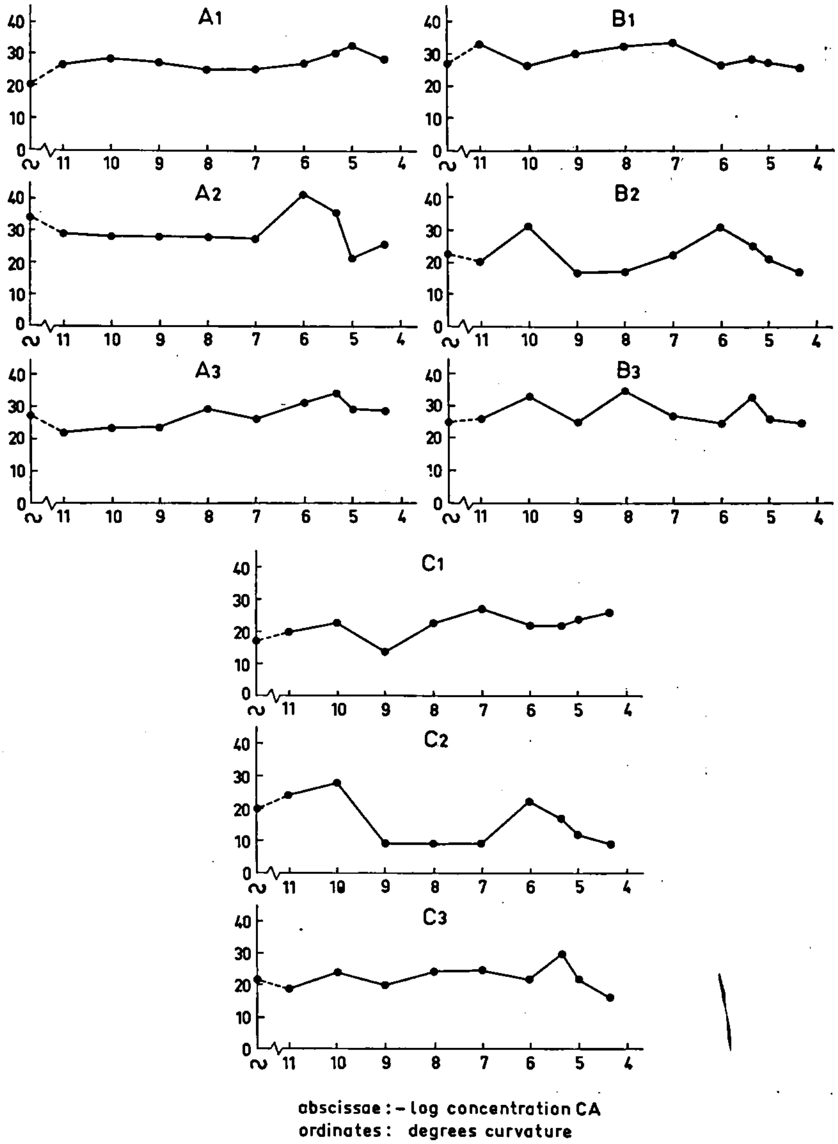

Fig. 2. The epinastic response of petioles of different leaf pairs to caffeic acid in darkness.

Plants grown in summer.

A: petioles of the fourth leaf pair

B: petioles of the fifth leaf pair

C: petioles of the sixth leaf pair

(Exp. nrs. 1, 2 and 3 from Table 2)

TABLE 2

The epinastic response of petioles of the fourth, fifth and sixth leaf pairs to caffeic acid in darkness

Plants grown in summer

exp. no.	concentration caffeic acid	degrees curvature		
		4th leaf pair	5th leaf pair	6th leaf pair
1	0	20 ± 1 (10)	27 ± 2 (10)	17 ± 3 (10)
	10^{-11}	26 ± 2 (10)	33 ± 4 (10)	20 ± 3 (10)
	10^{-10}	28 ± 2 (10)	26 ± 2 (10)	23 ± 3 (10)
	10^{-9}	27 ± 3 (10)	30 ± 3 (10)	14 ± 3 (10)
	10^{-8}	25 ± 2 (10)	32 ± 5 (10)	23 ± 5 (10)
	10^{-7}	25 ± 1 (10)	33 ± 4 (10)	27 ± 4 (10)
	10^{-6}	27 ± 2 (10)	26 ± 3 (10)	22 ± 3 (10)
	5×10^{-6}	30 ± 2 (10)	28 ± 3 (10)	22 ± 3 (10)
	10^{-5}	32 ± 2 (10)	27 ± 3 (10)	24 ± 6 (10)
	5×10^{-5}	28 ± 3 (10)	25 ± 2 (10)	26 ± 4 (10)
2	0	34 ± 3 (10)	22 ± 4 (10)	19 ± 3 (10)
	10^{-11}	29 ± 2 (10)	20 ± 4 (10)	24 ± 6 (10)
	10^{-10}	28 ± 2 (10)	31 ± 5 (10)	28 ± 4 (10)
	10^{-9}	28 ± 3 (10)	17 ± 4 (10)	9 ± 3 (10)
	10^{-8}	28 ± 2 (10)	17 ± 5 (10)	9 ± 5 (10)
	10^{-7}	27 ± 2 (10)	22 ± 2 (10)	9 ± 4 (10)
	10^{-6}	41 ± 3 (10)	31 ± 3 (10)	22 ± 3 (10)
	5×10^{-6}	35 ± 5 (10)	25 ± 5 (10)	17 ± 5 (10)
	10^{-5}	21 ± 2 (10)	21 ± 3 (10)	12 ± 4 (10)
	5×10^{-5}	25 ± 3 (10)	17 ± 2 (10)	9 ± 2 (10)
3	0	27 ± 2 (10)	25 ± 3 (10)	21 ± 4 (10)
	10^{-11}	22 ± 2 (10)	26 ± 2 (10)	19 ± 3 (10)
	10^{-10}	23 ± 2 (10)	33 ± 4 (10)	24 ± 2 (10)
	10^{-9}	23 ± 1 (10)	25 ± 6 (10)	19 ± 1 (10)
	10^{-8}	29 ± 3 (10)	35 ± 4 (10)	24 ± 2 (10)
	10^{-7}	26 ± 2 (10)	27 ± 3 (10)	25 ± 3 (10)
	10^{-6}	31 ± 3 (10)	25 ± 2 (10)	23 ± 4 (10)
	5×10^{-6}	34 ± 4 (10)	33 ± 4 (10)	30 ± 3 (10)
	10^{-5}	29 ± 3 (10)	26 ± 3 (10)	22 ± 5 (10)
	5×10^{-5}	28 ± 4 (10)	25 ± 4 (10)	16 ± 2 (10)

TABLE 3

The epinastic response of petioles of the third leaf pair to indoleacetic acid, in light and in darkness

Plants grown in winter

concentration indoleacetic acid	degrees curvature	
	in light	in darkness
0	20 ± 6 (10)	22 ± 5 (9)
10^{-9}	22 ± 4 (9)	28 ± 5 (10)
10^{-8}	21 ± 3 (8)	44 ± 6 (10)
10^{-7}	59 ± 13 (10)	47 ± 7 (10)
10^{-6}	100 ± 16 (10)	81 ± 9 (10)

TABLE 4

The epinastic response of petioles of the third leaf pair to gibberellic acid, in light and in darkness

Plants grown in winter

concentration gibberellic acid	degrees curvature	
	in light	in darkness
0	23 ± 5 (10)	21 ± 4 (8)
10^{-9}	15 ± 2 (9)	15 ± 3 (10)
10^{-8}	15 ± 3 (10)	20 ± 4 (10)
10^{-7}	16 ± 3 (10)	16 ± 4 (10)
10^{-6}	15 ± 3 (9)	10 ± 2 (9)

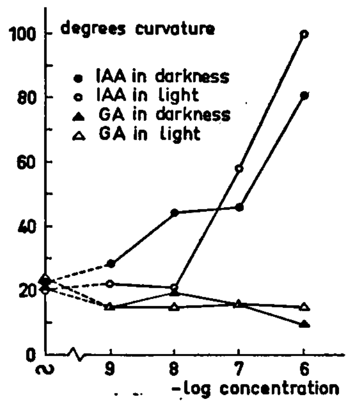

Fig. 3. A comparison of the effect of indoleacetic acid and gibberellic acid on the epinastic curvature.
Petioles of the third leaf pair of plants grown in winter.

The next step was to test the effect of caffeic acid in combination with indoleacetic acid. These results are given in Table 5 and Table 6 for the response of petioles of the third and those of the fifth leaf pair respectively. These results are given graphically in Figs. 4 and 5.

The combination of a series of concentrations of caffeic acid and indoleacetic acid shows a different picture of interaction at the different concentrations of indoleacetic acid tested.

DISCUSSION

The results reported in this paper make it clear that caffeic acid does not evoke a response in the isolated petioles of *Coleus* comparable to that elicited by indoleacetic acid (Fig. 3). Indoleacetic acid en-

TABLE 5

The epinastic response of petioles of the third leaf pair to caffeic acid alone or in combination with indoleacetic acid, in darkness

Plants grown in summer

concentration indoleacetic acid	concentration caffeic acid	degrees curvature	
		caffeic acid alone	caffeic acid + indoleacetic acid
10^{-11}	0	26 ± 2 (10)	19 ± 2 (10)
10^{-11}	10^{-11}	23 ± 3 (10)	16 ± 3 (10)
10^{-11}	10^{-10}	25 ± 3 (10)	20 ± 2 (10)
10^{-11}	10^{-9}	22 ± 3 (10)	14 ± 2 (10)
10^{-11}	10^{-8}	18 ± 3 (10)	24 ± 2 (10)
10^{-11}	10^{-7}	21 ± 2 (10)	19 ± 4 (10)
10^{-10}	0	24 ± 3 (10)	19 ± 2 (8)
10^{-10}	10^{-11}	21 ± 5 (10)	28 ± 3 (8)
10^{-10}	10^{-10}	24 ± 3 (10)	23 ± 2 (8)
10^{-10}	10^{-9}	19 ± 3 (10)	27 ± 3 (8)
10^{-10}	10^{-8}	28 ± 2 (10)	26 ± 3 (10)
10^{-10}	10^{-7}	27 ± 3 (10)	27 ± 3 (10)
10^{-9}	0	35 ± 3 (10)	27 ± 3 (10)
10^{-9}	10^{-11}	27 ± 3 (10)	31 ± 3 (10)
10^{-9}	10^{-10}	26 ± 3 (10)	29 ± 4 (10)
10^{-9}	10^{-9}	30 ± 2 (10)	28 ± 3 (10)
10^{-9}	10^{-8}	27 ± 2 (10)	29 ± 4 (10)
10^{-9}	10^{-7}	33 ± 3 (10)	38 ± 3 (10)
10^{-8}	0	26 ± 4 (10)	38 ± 2 (10)
10^{-8}	10^{-11}	30 ± 3 (10)	32 ± 3 (10)
10^{-8}	10^{-10}	23 ± 2 (10)	38 ± 3 (10)
10^{-8}	10^{-9}	23 ± 3 (10)	41 ± 4 (10)
10^{-8}	10^{-8}	28 ± 3 (10)	44 ± 6 (10)
10^{-8}	10^{-7}	23 ± 3 (10)	43 ± 5 (10)
10^{-7}	0	31 ± 6 (10)	47 ± 6 (8)
10^{-7}	10^{-11}	37 ± 4 (10)	51 ± 7 (6)
10^{-7}	10^{-10}	23 ± 5 (10)	45 ± 5 (6)
10^{-7}	10^{-9}	27 ± 2 (10)	55 ± 6 (6)
10^{-7}	10^{-8}	27 ± 4 (10)	48 ± 4 (10)
10^{-7}	10^{-7}	23 ± 4 (10)	48 ± 8 (10)

hances the epinastic curvature in concentrations above 10^{-7}. In concentrations between 10^{-9} and 10^{-7} there is an effect observable (SOEKARJO, 1961): there is a concentration dependent *decrease* of the curvature, in some cases preceded by an increase.

Under the same experimental conditions gibberellic acid does not promote the epinastic curvature: there is either no effect or an inhibition.

Although the epinastic response to caffeic acid is not as clear as that to indoleacetic acid and gibberellic acid respectively, some interesting points might be put forward.

TABLE 6

The epinastic response of petioles of the fifth leaf pair to caffeic acid alone or in combination with indoleacetic acid, in darkness

Plants grown in summer

concentration indoleacetic acid	concentration caffeic acid	degrees curvature	
		caffeic acid alone	caffeic acid + indoleacetic acid
10^{-11}	0	30 ± 6 (10)	21 ± 3 (10)
10^{-11}	10^{-11}	30 ± 2 (10)	20 ± 4 (10)
10^{-11}	10^{-10}	31 ± 4 (10)	15 ± 3 (10)
10^{-11}	10^{-9}	26 ± 3 (10)	30 ± 5 (10)
10^{-11}	10^{-8}	20 ± 3 (10)	27 ± 4 (10)
10^{-11}	10^{-7}	23 ± 3 (10)	25 ± 4 (10)
10^{-10}	0	23 ± 4 (10)	17 ± 3 (8)
10^{-10}	10^{-11}	29 ± 5 (10)	26 ± 6 (8)
10^{-10}	10^{-10}	16 ± 2 (10)	27 ± 5 (8)
10^{-10}	10^{-9}	19 ± 3 (10)	22 ± 5 (8)
10^{-10}	10^{-8}	19 ± 2 (10)	22 ± 5 (8)
10^{-10}	10^{-7}	18 ± 4 (10)	20 ± 3 (10)
10^{-9}	0	20 ± 5 (10)	25 ± 4 (10)
10^{-9}	10^{-11}	21 ± 3 (10)	20 ± 3 (10)
10^{-9}	10^{-10}	16 ± 2 (10)	23 ± 2 (10)
10^{-9}	10^{-9}	27 ± 4 (10)	17 ± 3 (10)
10^{-9}	10^{-8}	16 ± 5 (10)	15 ± 3 (10)
10^{-9}	10^{-7}	22 ± 4 (10)	23 ± 3 (10)
10^{-8}	0	24 ± 3 (10)	30 ± 2 (10)
10^{-8}	10^{-11}	22 ± 2 (10)	27 ± 3 (10)
10^{-8}	10^{-10}	14 ± 4 (10)	26 ± 3 (10)
10^{-8}	10^{-9}	13 ± 3 (10)	32 ± 6 (10)
10^{-8}	10^{-8}	14 ± 4 (10)	17 ± 5 (10)
10^{-8}	10^{-7}	21 ± 2 (10)	34 ± 5 (10)
10^{-7}	0	20 ± 3 (10)	39 ± 5 (8)
10^{-7}	10^{-11}	19 ± 3 (10)	46 ± 5 (6)
10^{-7}	10^{-10}	31 ± 6 (10)	52 ± 7 (6)
10^{-7}	10^{-9}	26 ± 3 (10)	45 ± 6 (6)
10^{-7}	10^{-8}	24 ± 3 (10)	36 ± 4 (10)
10^{-7}	10^{-7}	24 ± 3 (10)	39 ± 5 (10)

We see (Fig. 1) that the curvature *vs.* concentration curve for plants grown in winter, shows two small maxima and a clear promotion of the curvature by light at all the concentrations tested. This type of response is not present in summer-grown plants. The promotion by light is no more evident. The concentrations of caffeic acid that do enhance the curvature in winter-grown plants do not always do so in summer-grown plants. In some instances only at concentrations above 10^{-7} an enhancement of the curvature is to be seen, in other cases only at concentrations below 10^{-7}. Even inhibition occurred (Fig. 2).

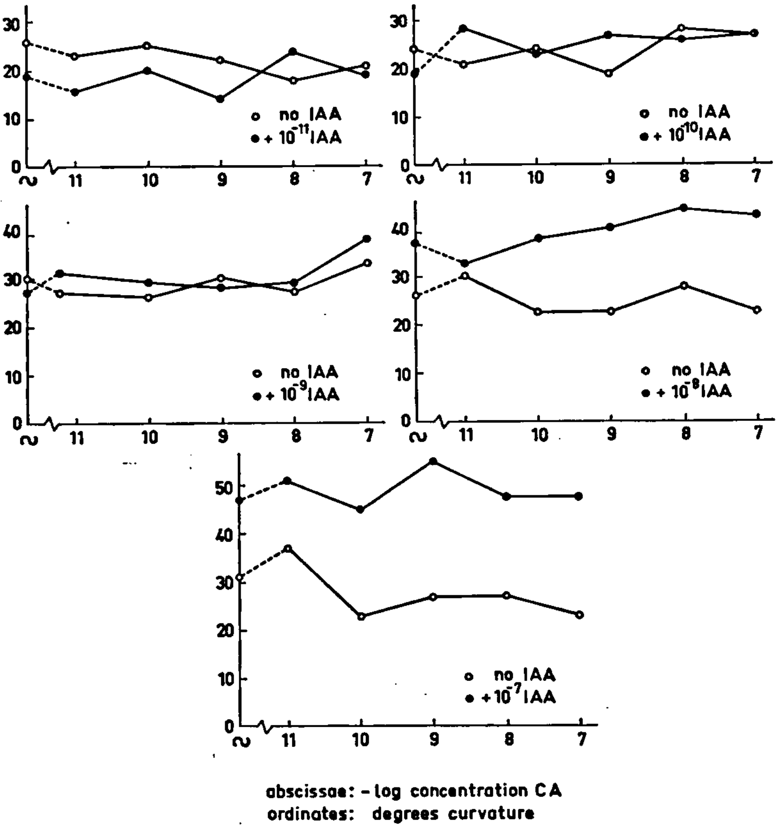

Fig. 4. The epinastic response of petioles of the third leaf pair to caffeic acid alone or in combination with indoleacetic acid, in darkness.
Plants grown in summer.

The difference in response between plants grown in winter and those grown in summer, suggest a light-dependent internal level of caffeic acid or related compounds, further complicated by differences in the level of endogenous auxin. This might be due to the fact that the plants were grown in the greenhouse with a supplemented day-length in winter, not with a constant light intensity during the whole of the year.

As the epinastic response to caffeic acid is not suggestive of a direct growth substance activity, it is tempting to try and explain the effects by an interaction with indoleacetic acid destructing enzyme systems.

Caffeic acid is mentioned in SPECTOR *et al.* (1956) as a biological antioxidant, a substance having "antioxygenic activity in vivo or in vitro".

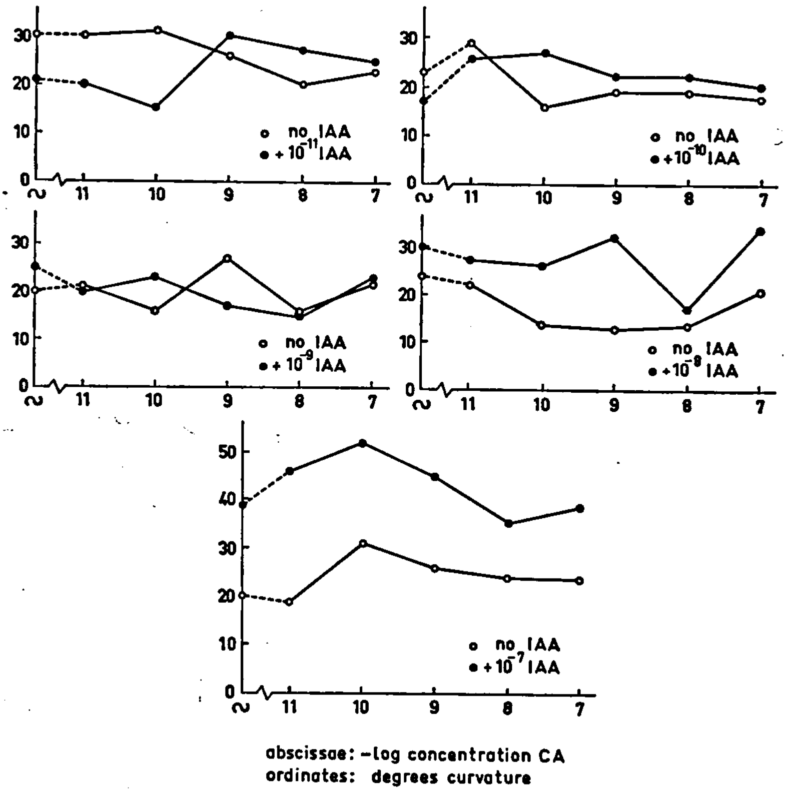

Fig. 5. The epinastic response of petioles of the fifth leaf pair to caffeic acid alone or in combination with indoleacetic acid, in darkness.
Plants grown in summer.

Elema (1960) and Kögl & Elema (1960) discussed the "auxin sparing" activity of gibberellic acid in the light of a promotion of the production of caffeic acid and related compounds inhibiting the activity of indoleacetic acid oxidase systems. Recently more evidence in this direction has accumulated, see e.g. Engelsma (1964), Konings (1964) and Tomaszewski (1964).

The promotion of the epinastic curvature in concentrations of caffeic acid above 10^{-7} might indeed be caused by an inhibition of the destruction of the endogenous growth substance present in the petioles.

The increase of the epinastic curvature with increasing concentrations of indoleacetic acid, is interrupted by a decrease in the region between 10^{-9} and 10^{-7} (Soekarjo, 1961). The effect of lower concentrations of caffeic acid and the interaction with added indoleacetic acid might be explained by an "auxin sparing" action interfering with this typical response towards indoleacetic acid. So it is still possible that even in this lower region of concentrations of caffeic acid, the effect is of an "auxin sparing" nature, the auxin being endogenous and/or added.

The promotion of the epinastic curvature caused by indoleacetic acid in a concentration of 10^{-8} by a further addition of caffeic acid in concentrations below 10^{-8} (Fig. 4), the only clear case of a promotion, might be seen as an indication of the mechanism. The concentration of indoleacetic acid of 10^{-8} might be the critical concentration, because of the fact that near this concentration the curvature *vs.* concentration curve has its minimum.

Although the mechanism of the interference of caffeic acid with the development of the epinastic curvature in petioles of *Coleus* has not been definitely explained, contrary to the view of VENDRIG (1961), the present author is inclined to regard caffeic acid as a minor stimulator, but not as a growth substance. by its own rigt. Nor do the results obtained with *Coleus* substantiate the more recent explanation of the effects of the group of hydroxycinnamic acid as "true auxins, though possibly as weak ones" (BUFFEL & VENDRIG, 1963).

It is much more probable that indoleacetic acid is the endogenous growth substance in *Coleus scutellarioides* (= *C. rhenaltianus* in Vendrig's papers), as has been established for *C. blumei* by SCOTT & JACOBS (1964).

The response towards gibberellic acid might be related to the level of the endogenous compounds inhibiting the activity of indoleacetic acid destructing enzyme systems. If the activity of gibberellic acid, as ELEMA (1960) supposes, is an activation of the production of this type of compounds, a high level of these compounds in the plant diminishes the effect and may even lead to supra-optimal concentrations of the endogenous growth substance in the plant tissue.

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