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Antagonism of Some Gibberellin Actions by a Substituted Pyrimidine[1]

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ABSTRACT

From a comparison of the effects of seven growth retardants and abscisic acid (ABA) on various growth systems, it was found that the gibberellin-regulated growth of lettuce hypocotyls was uniquely inhibited by the growth retardant, α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol (EL-531). Auxin-regulated growth of coleoptile sections was inhibited by Phosfon and only slightly by EL-531 and Alar. Cytokinin-regulated growth of *Xanthium* cotyledons showed little or no inhibition by any of the retardants. ABA was inhibitory in all three types of tests. The distinctive effects of EL-531 against gibberellin-stimulated growth and the general ability of gibberellic acid to relieve EL-531 inhibition suggest that this retardant acts in part against the gibberellin-stimulated growth system, but at a locus which discriminates between growth and nongrowth functions of gibberellic acid. It shows little or no antagonism of gibberellin actions which do not involve growth: the barley endosperm test and the *Rumex* leaf senescence test.

The advent of effective growth retardants over the last several years has provided not only new possibilities for artificially controlling plant growth, but also possibilities for analyzing the endogenous controls of plant growth. The most effective retardants include certain phosphoniums, hydrazides, substituted fluorenes, and several types of quaternary ammonium compounds including carbamates, cholines, and piperidines. Several of these types are effective inhibitors of gibberellin biosynthesis (1, 13). Growth retardations by all six classes of retardants have been reported to be relieved by the addition of gibberellin. A novel type of growth retardant has recently been reported by scientists of Eli Lilly and Company which is a substituted pyrimidine methanol, (α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol, or EL-531) (20). In an effort to compare the actions of the various growth retardants, their activities against each of the growth regulator systems which drive growth have been tested.

METHODS

The growth retardants which were tested include 2,4-dichlorobenzyltributylphosphonium chloride (Phosfon-D, or CBBP),[2] (2-chloroethyl)trimethyl-ammonium chloride (Cycocel, CCC, or chlormequat), succinic acid-2,2-dimethylhydrazide (Alar, B-nine, or SADH), ammonium(5-hydroxycarvacryl)-trimethyl chloride piperidine carboxylate (Amo-1618, or ACPC), α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol (EL-531), and two commercial materials of unknown structure: UNI-529 (a product of the Uniroyal Company, and a derivative of succinic acid hydrazide) and BAS-0660 (a product of the BASF Corporation, and a derivative of choline). Abscisic acid was included for comparison as a natural growth inhibitor.

The bioassays utilized were the oat coleoptile assay for auxin-stimulated growth, the *Xanthium* cotyledon test for cytokinin-stimulated growth (4), the lettuce hypocotyl test (5), the barley seed test (9), and the *Rumex* leaf disc test for gibberellin responses (21).

RESULTS

In order to compare the effectiveness of the various growth inhibitors on gibberellin-regulated growth, serial dilutions of each compound were tested for their ability to inhibit the lettuce hypocotyl growth in the presence of 10 μM GA. Comparative effects are shown in Figure 1, where a 50% inhibition of growth was obtained with EL-531 at about 10 μM, with ABA at about 0.1 mM, and the other growth retardants were without effect on this system. The ability of EL-531 to inhibit the gibberellin-activated elongation of the lettuce seedling is distinctive among the growth retardants tested; only ABA shares in this action.

A similar comparison of growth inhibitors on auxin-stimulated growth is obtained using the *Avena* straight growth test. In Figure 2, the various inhibitors were added to the medium (2% sucrose, 20 mM KH_2PO_4, 100 mg/liter $MnCl_2$) with 10 μM indoleacetic acid. In this system, it can be seen that the ABA inhibition of growth, reported initially by Thomas *et al.* (19), is the strongest of the compounds tested here; of the various growth retardants tested, only CBBP showed a strong inhibition, and weak effects were found for EL-531 and ACPC.

A comparison of the effects of the growth inhibitors on cytokinin-stimulated growth was obtained using the *Xanthium* cotyledon test. A representative test is shown in Figure 3, where it can be seen that, in the presence of 10 μM benzyladenine (BA), ABA is again outstanding in its effectiveness, reaching 50% inhibition at about 10 μM. A lesser inhibition is observed for EL-531. The other growth retardants showed little or no inhibition of growth in this system.

From these assays it appears that most growth retardants tested are ineffective in suppressing the activities of the three major growth-stimulating systems in plants, *i.e.*, auxin, gibberellin, and cytokinin. Abscisic acid shows activity against each of these regulatory systems, EL-531 shows a particular

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[2] Abbreviations: CBBP: 2,4-dichlorobenzyltributylphosphonium chloride; CCC: (2-chloroethyl)trimethylammonium chloride; SADH: succinic acid-2,2-dimethylhydrazide; ACPC: ammonium (5-hydroxycarvacryl)-trimethyl chloride piperidine carboxylate; EL-531; α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidine methanol; BA: benzyladenine.

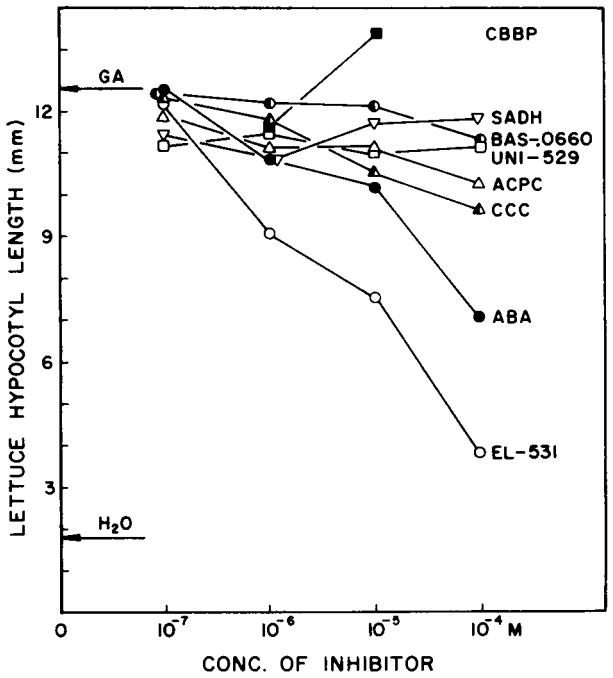

FIG. 1. The effects of seven growth retardants and ABA on the gibberellin-stimulated growth of the lettuce hypocotyl. GA at 10 μM was included in each solution. Ten seedlings per treatment were used.

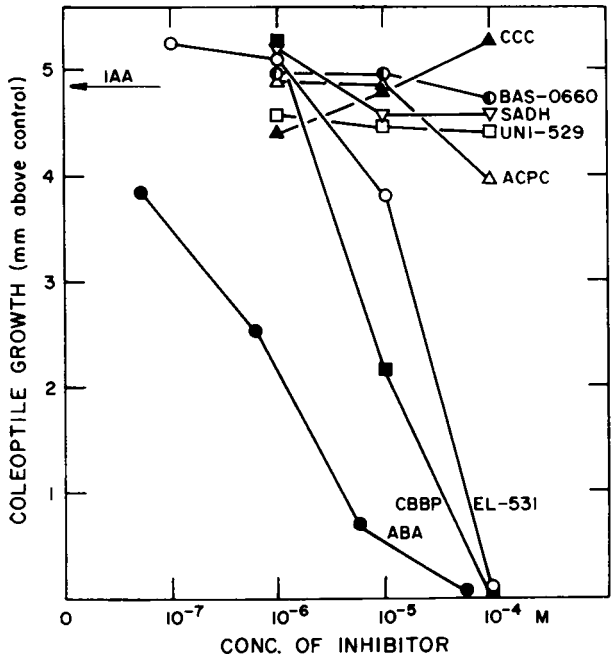

FIG. 2. The effects of growth retardants and ABA on the auxin-stimulated growth of the oat coleoptile section. Each solution contained 10 μM IAA. Ten 5-mm sections were used per treatment.

effectiveness against the gibberellin-stimulated growth, and CBBP is effective in inhibiting auxin-stimulated growth.

A natural question would be to ask whether the EL-531 inhibition of the lettuce hypocotyl test could be relieved by further additions of GA. A test of this is illustrated in Figure 4, where it can be seen that in the presence of inhibiting concentrations of EL-531 increases in GA can bring about increases in growth. The dwarfing effects of EL-531 on seedlings are generally overcome by the application of GA; this effect is illustrated with corn seedlings grown in the greenhouse, where seed treatment with talc powder containing 1%

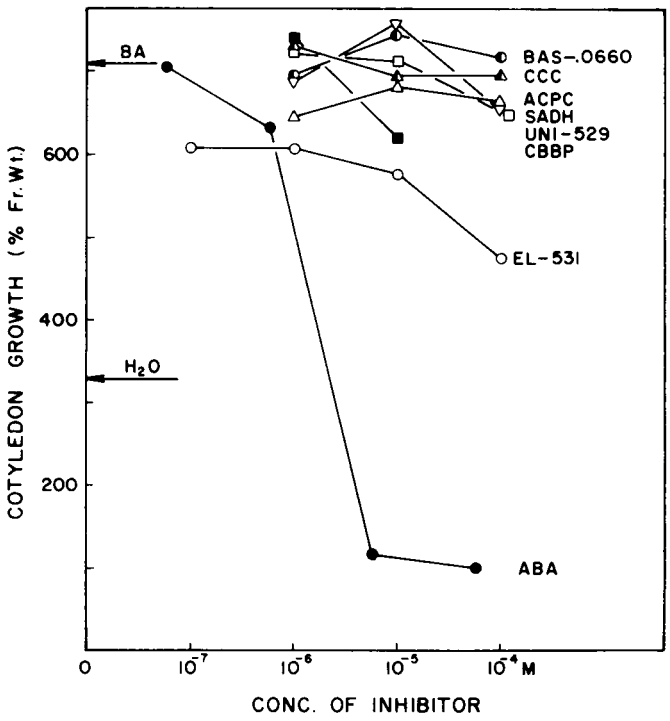

FIG. 3. The effects of growth retardants and ABA on the cytokinin-stimuated growth of the *Xanthium* cotyledon. Each solution contained 10 μM BA. Ten cotyledon pieces were used per treatment.

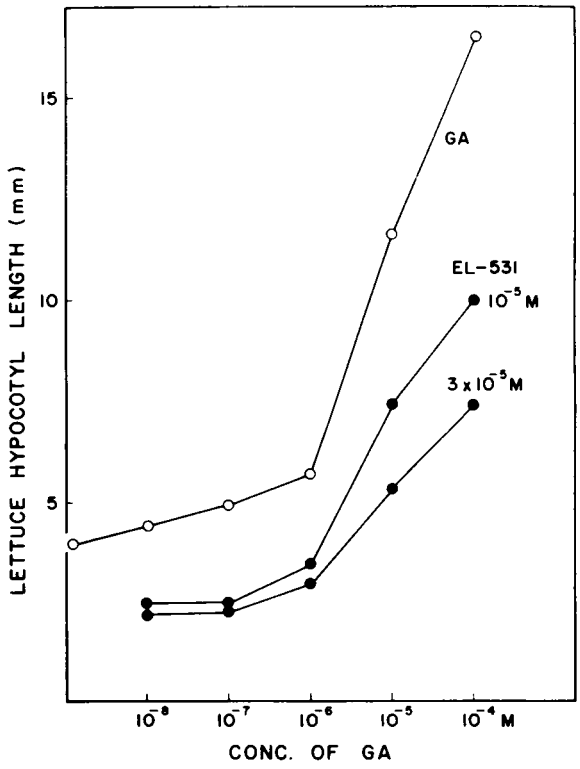

FIG. 4. Stimulation of lettuce hypocotyl growth by GA in the presence of two concentrations of the growth retardant EL-531.

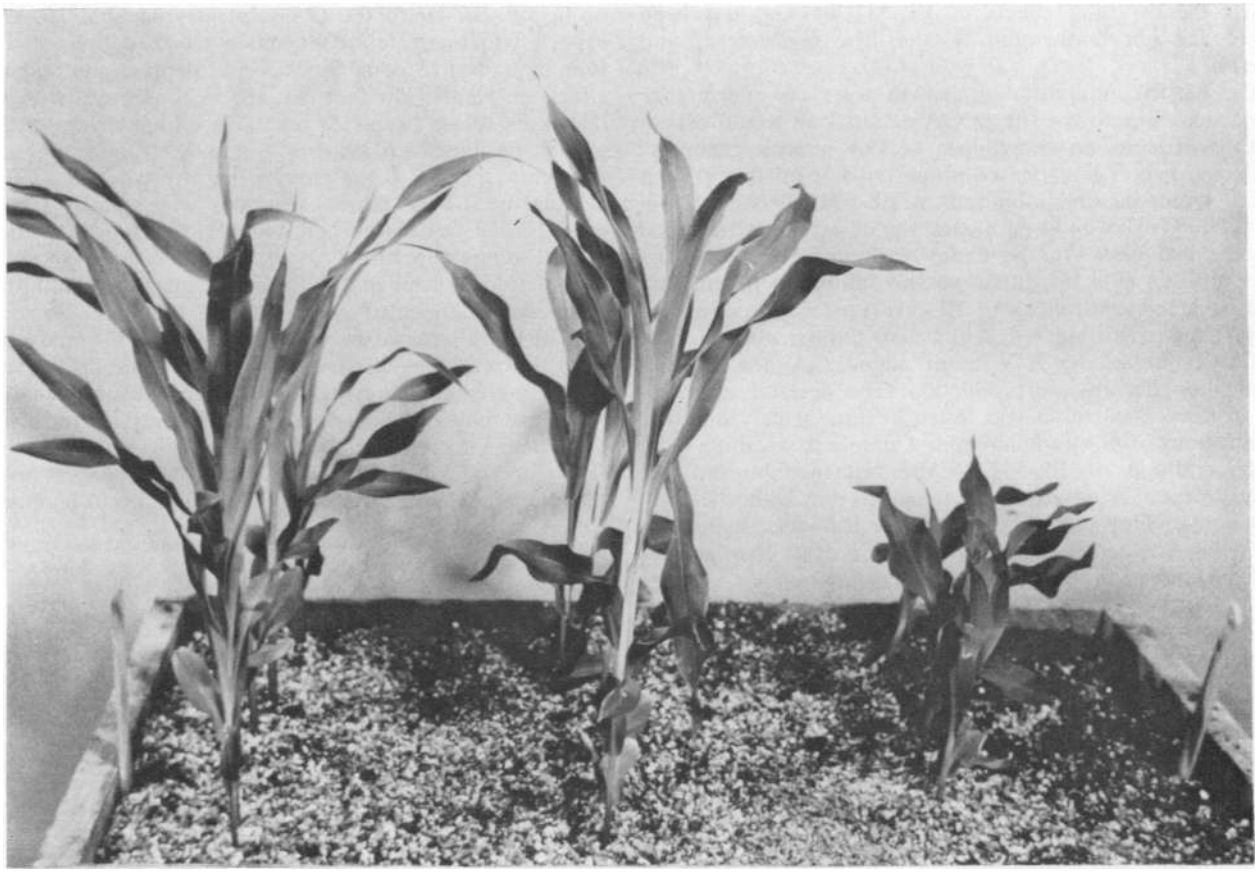

FIG. 5. The dwarfing effects on EL-531 on corn seedlings after seed treatment with 1% dust of EL-531, and the relief of the dwarfing effects with GA (0.2 ml of 0.1 mM per plant, two applications). Left to right: controls, EL-531 followed by GA, EL-531 only.

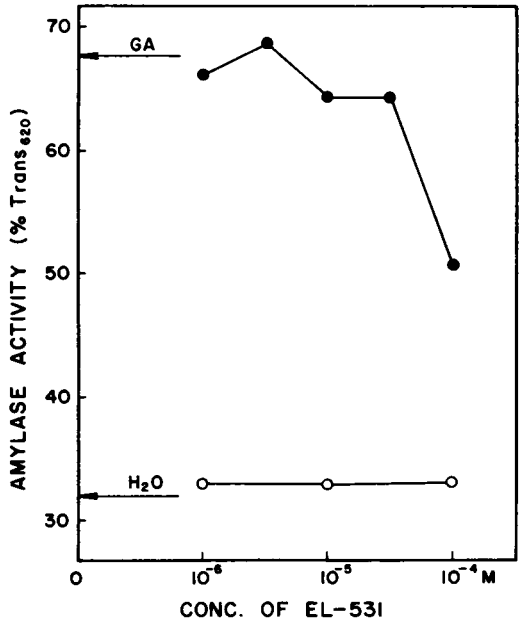

FIG. 6. The effect of EL-531 on the amylase release by barley endosperm. Averages are of 10 separate tests, 3 replicates of 5 seeds each with or without added GA (0.1 μM).

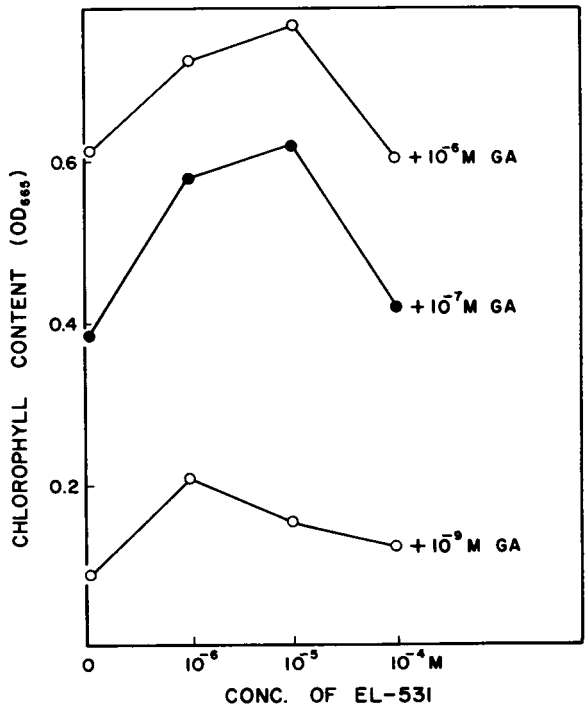

FIG. 7. The effect of EL-531 on the senescence of *Rumex* leaf discs as evidenced by chlorophyll content. Five discs were used per treatment.

EL-531 produced dwarf corn plants as shown in Figure 5, and weekly applications of 0.2 ml of 0.1 mM GA completely restored the corn plants to normal growth. Similar

relief of the dwarfing effects of EL-531 by GA has been observed for chrysanthemum, Easter lily, *Hydrangea*, and *Poinsettia*.

If the EL-531 inhibition of growth were an interference of some step close to the site of GA action, one would expect that it could show an antagonism of GA actions generally. We selected two GA responses which did not involve growth stimulations for possible inhibition by EL-531: the stimulation of amylase in barley endosperm, and the deferral of senescence in *Rumex* leaf discs. In the barley endosperm test with 0.1 μM GA present (Fig. 6), there was no inhibition of amylase formation at concentrations of EL-531 up to 30 μM; an inhibitory effect was observed at 0.1 mM, though not reaching the 50% inhibition level. Without added GA, the EL-531 did not alter the amylase production. The deferral of senescence in *Rumex* leaf discs was tested at three widely different concentrations of GA ranging from 1 nmole to 1 μmole (Fig. 7). The addition of EL-531 to the test medium did not antagonize the GA deferral of senescence, but rather slightly increased the chlorophyll content of the leaf disc. In neither test wherein GA action regulated an action other than growth did EL-531 effectively antagonize the GA function.

DISCUSSION

The plant growth retardants, or chemicals which can restrict the elongation growth of plants without formative effects (1), might be expected to interfere with the functioning of one of the growth regulator systems which are known to actuate plant growth. The experiments reported here permit a comparison of the effects of seven growth retardants on gibberellin-induced growth, auxin-induced growth, and cytokinin-induced growth; the results indicate that most retardants do not appreciably inhibit growth in the three systems examined, but that there are several interesting exceptions. EL-531 was found to interfere strongly with gibberellin-induced growth in the lettuce test, CBBP was found to inhibit auxin-induced growth in the coleoptile test (see also Ref. 3), and lesser inhibitions were observed for ACPC and EL-531 in this assay. In contrast to the growth retardants, ABA was found to inhibit all three types of growth.

The growth inhibitions by retardants are generally relieved by applications of GA (1, 13). Several types of growth retardants are known to suppress GA biosynthesis, including members of the choline, piperidine, and phosphonium classes of retardants (13). Hydrazide retardants may (22) or may not (8) suppress GA biosynthesis. At least one retardant, chlorflurenol, is a clear exception and has not been found to suppress GA biosynthesis (18). Experiments in this laboratory will be published elsewhere indicating that EL-531 does not suppress GA biosynthesis by the mold, *Fusarium moniliforme*. The ineffectiveness of the growth retardants as inhibitors of such GA actions as the stimulation of amylase formation in the barley endosperm (16) or the retardation of leaf senescence in *Rumex* (7) where gibberellin biosynthesis is not involved strengthens the idea that most retardant actions may result from an inhibition of GA biosynthesis. The special effectiveness of EL-531 in inhibiting growth in the presence of added GA (Fig. 1) implies that this retardant is distinctive in its retardant action, and that one component of its retardant effect may be an antagonism of a GA-stimulated function in growth.

Paleg *et al.* (16) described five possible modes of action of growth retardants as antagonists of GA function. A retardant may depress GA biosynthesis or the synthesis of a substrate with which GA must react, depress the binding of GA to the substrate, inactivate GA, or, finally, depress reactions which occur subsequent to the GA-substrate reaction. It would be expected that any retardant action which depressed the first four of these five possibilities would depress any and all GA effects on plants. The fact that EL-531 interferes with growth responses (Figs. 1 and 5) and does not interfere with non-growth responses to GA (Fig. 6 and 7) strongly suggests that this retardant may act in part by the fifth category of Paleg *et al.*; that is, it may depress reactions which occur subsequent to the initial GA action and which are entrained in the reactions leading to growth.

It should be kept in mind that not all growth retardant effects can be attributed directly to GA functions. Some effects of CBBP are relieved by auxin applications (3), and some effects of CCC may produce actual increases in GA content (17) or increases in growth (6). While the effects of any growth retardant may be through multiple actions in the plant, the distinctive effects of EL-531 on GA-enhanced growth reactions suggest that this retardant may be especially useful in the regulation and analysis of GA stimulations of plant growth.

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