661.18:581.143:576.32:581.1.036.5
635.65:631.811.98:632.938.1:632.111.5:58

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SURFACE-ACTIVE CHEMICALS AS REGULATORS OF PLANT GROWTH, MEMBRANE PERMEABILITY, AND RESISTANCE TO FREEZING

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1. INTRODUCTION

Modification of plant growth by surface-active organic chemicals is of considerable interest to plant physiologists and agronomists. Plant physiologists are interested in the mode of action of these chemicals as antimetabolites, in the induced change of permeability, and in the antagonistic action between these compounds and gibberellin (7). Of interest to both groups are the observed retardation of stem growth, the increase in green color of the leaves (7, 15) the increase in flower initiation of woody plants (36) and the increased resistance to water stress (15), to salinity and excessively high fertilizer application (21), to smog (31), and to frost (22). Most widely used in experiments are 'B-9', NN-dimethylsuccinichydrazide, and 'CCC', 2-chloroethyltrimethylammonium chloride, but many 'growth retardants' with widely different chemical structures have been found (see CATHEY, 1964, p. 274). The same is also true for auxins; e.g. compare the structure of isatin (14), indolacetic acid, and S-(carboxymethyl-dimethyl dithiocarbamate) (39). Evidently, steric factors, the strength of hydrogen bonds and VAN DER WAALS forces determine the physiological activity of such compounds (39).

Animal physiologists have also found a large number of widely different 'radiomimetic' compounds (3). The structure of 2-chloroethyldimethylamine, which stops malignant cell growth, closely resembles CCC. It is suggested that radiomimetic compounds function by a crosslinking mechanism involving a reaction with the amino, carboxyl, and sulfhydryl groups of proteins (12) and of nucleoproteins (11). If this is true, bifunctional compounds should be physiologically more effective than unifunctional compounds, and indeed BERG et al. (4) demonstrated that 1,5-difluoro-2,4-dinitrobenzene protected red blood cells against lysis in distilled water, while 1-monofluoro-2,4-dinitrobenzene was ineffective. The protection against lysis is comparable with the protection of plant cells against frost. In both cases the treated cytoplasmic membrane is more stable and can stand the exerted mechanical stress.

Bifunctional radiomimetic compounds can cross-link proteins, but not every cross-linking agent is biologically active (3). The great specificity of the proteins of plant cells also explains why there is no universal chemical growth regulator for all kinds of plants (7).

2. EXPERIMENTAL APPROACH

The following 4 groups of chemicals were chosen for the experiments described in this paper: compounds with different lengths of the hydrocarbon chain (1), carbonamides of decenylsuccinic acid (2), acetylated compounds (3), and fluorinated compounds (4).

1. *Compounds with different length of the hydrocarbon chain:* 2-alkenylsuccinic acids, $CH_3(CH_2)_n.CH{:}CH.CH_2.CH(COOH)CH_2.COOH$; alkyltrimethylammoniumbromides, $CH_3(CH_2)_n.N(CH_3)^+_3.Br^-$, alkylimidazolines, $CH_3(CH_2)_n.C{:}CH.NH.CH{:}N$, and alkylmethylsulfoxides, $CH_3\,(CH_2)_n.\,SO.CH_3$.

Long chain compounds are more effective as crosslinking agents than short chain compounds. Even a unifunctional compound such as heptylbromide may be effective in some cases (3). The additional hydrogen bonds may be formed around free hydrocarbon chains because of the lack of competition for such bonds on the hydrocarbon chain itself (23). Such bonds may also increase the stability of the protein against denaturation as shown by BALLOU et al. (5, 6). Serum albumin is protected against heat denaturation by fatty acid salts, the protection being more effective with increasing number of C-atoms of the compound.

Alkenylsuccinic acids increase permeability of cell membranes to water, probably by incorporation of the molecules into the lipid layer of the plasma membrane (17). The increase in permeability with increasing number of CH_2-groups is probably due to greater lipid solubility of the compounds. The above chemicals of different chain length were tested for their effect on the water permeability of bean roots.

Decenylsuccinic acid also retards leaf growth of young bean plants (18). This effect on leaf growth generally disappears after 1 or 2 weeks, probably because the compound is metabolized (19). A treatment with CCC or B-9,

however, often is still effective half a year or a year later (30). In many situations, compounds which are easily metabolized are desirable since often only a temperary effect (e.g. protection against night frost) is needed and thereafter normal growth is desired. Long-chain compounds are attractive for this purpose.

2. *Carbonamides of decenylsuccinic acid:* decenylsuccinamic acid, decenyl-NN-dimethylsuccinamic acid, decenylsuccinichydrazide, decenyl-NN-dimethylsuccinichydrazide, and decenylsuccinicdihydrazide.

Surface-active agents are classified by NASH (23) according to the kind of hydrogen bond they form. This classification seems to accord better with biological effects than does the one based on ionic status. Hydrophilic compounds have the ability to form strong H-bonds; therefore they are biologically most active. Hydrogen bonds between a protein molecule and a cross-linking agent may be formed by acceptance of the lone pair electrons of the N-atom of an amino group of the agent. Addition of methyl groups to the N-atom ($-N(CH_3)_2$) will increase the H-bonding capacity of the compound. Thus, an analysis of decenylsuccinic acid and its monoamides (amido-, dimethylamido-, hydrazide-, and dimethylhydrazide-) seemed profitable. In this connection the effect of pH was also important, since the hydrogen bonding properties of such compounds depend on the concentration of the H^+ion (23). In addition, the effect of a bifunctional compound (decenylsuccinicdihydrazide) was compared with a unifunctional compound (decenylsuccinichydrazide). Finally, the effect of the chain length may be important; it can be studied with decenyldimethylsuccinichydrazide which is essentially B-9 with a chain of 10 C-atoms attached to it.

The effect of these compounds on growth of bean plants was studied at different pH, while also their effect on growth of excised cotyledons of *Cucurbita ficifolia* was measured. Growth of excised cotyledons of *Cucurbita pepo* is stimulated by addition of kinetin (20). I observed this phenomenon also in *Cucurbita ficifolia* after treatment with kinetin but also after treatment with decenylsuccinic acid. Compounds like B-9 and CCC generally retard growth, but sometimes, e.g. in cold weather, growth of treated plants greatly exceeds that of the control plants (22).

Decenylsuccinic acid gave no protection to early frost in strawberry flowers. The amides of this compound were tested on possible protection of strawberry flowers against frost.

3. *Acetylated compounds:* glycerol mono-, di-, and tri-acetate, glucose-pentaacetate, sucroseoctaacetate, and acetylated monoesters (glyceryl-, mannitol-, and sucrose-) of decenylsuccinic acid.

AGOSTINI and SCHULMAN (1) studied water transport across a phospholipid-protein barrier as a model for a cell membrane. Addition of the polysaccharide heparin blocked water transfer, while addition of chondroitin, an acetylated polysaccharide, greatly enhanced the water flux. They suggested that water transport across the plasmamembrane may be governed by the degree of acetylation of the polysaccharide attached to the membrane. Considering this suggestion, tests were made of the effect of acetylated glycerol and acetylated sugars on

water permeability and electrolyte permeability of root cell membranes, growth retardation of young bean plants, and growth stimulation of excised *Cucurbita* cotyledons. Water permeability was only slightly increased by these compounds and electrolyte permeability was used as a more sensitive test. Increase in electrolyte permeability is already measurable at concentration of the surface-active material, where no stimulatory effect on water permeation is yet observed (24).

Glyceroltriacetate, sucroseoctaacetate and the acetylated monoglycerylester of decenylsuccinic acid were sprayed on potato plants in the field to see if they had any effect on tuber yield. DYSON (9) observed that CCC caused earlier formation of tubers.

4. *Fluorinated compounds:* 1,5-difluoro-2,4-dinitrobenzene and 1-fluoro-2,4-dinitrobenzene. These reagents react by displacement of fluorine with amino-, sulfhydryl, tyrosyl, or histidyl groups of proteins to form dinitrophenyl derivatives. The monofluoro compound reacts with one such group, but the difluoro compound may react with two such groups, provided they are about 5 Å apart to form a dinitrophenylene cross-link (4). Tests with these 2 compounds were performed on water permeability, growth of young bean plants and frost resistance of beans.

3. MATERIAL AND METHODS

a. Chemicals.

If not mentioned otherwise, chemicals were bought commercially.

Samples of 2-alkenylsuccinic acids, including decenylsuccinic acid, and the monomethyl- and monoglycerylesters of this compound, were kindly provided by DR. B.J. HUMPHREY, Humphrey Chemical Inc. North Haven, Conn., U.S.A. Miss Barbara WOODING, Department of Botany and Plant Pathology, The Connecticut Agricultural Experiment Station, similarly provided me with samples of alkyltrimethylammoniumbromides and of alkylimidazolines. The synthesizing Laboratory of Am. Chem. Products Inc., Ambler, Pa., U.S.A. prepared the following chemicals for me: hexyl-, octyl-, and decyl-methylsulfoxide, decenylsuccinamic acid, decenyl-N,N-dimethylsuccinamic acid, and decenyl-NN-dimethylsuccinichydrazide. Decenylsuccinichydrazide and decenylsuccinicdihydrazide were synthesized by myself at the Organic Chemistry Department of the Agricultural University at Wageningen as follows:

Decenylsuccinichydrazide: 7 grams of the monomethyldecenylsuccinate were refluxed for 6 hours with 10 gram hydrazine hydrate in 40 ml absolute ethanol. The liquid was acidified with acetic acid to pH 6 and poured in excess 80% ethanol for cristallization. The precipitate was recristallized twice. The crystals were dried over $CaCl_2$ in a vacuum exsiccator. Melting point was 154 °C. C-content was 59.59% (theoretically 62.19%) and H-content 9.48% (9.70%). Possibly some crystal water is still in the preparation. The C-content of the monohydrate is theoretically 58.30% and that of the $\frac{1}{2}$ H_2O-hydrate 60.19%.

Decenylsuccinicdihydrazide: first dimethyldecenylsuccinate was prepared. 50 Grams of decenylsuccinic acid were refluxed for 22 hours in 120 ml absolute methanol, while 7 ml concentrated H_2SO_4 was added. Excess methanol was removed by destillation. The residue was shaken with 250 ml ice water. The water was washed 4 times with ether. Subsequently, the ether extracts were washed with 50 ml of H_2O, 50 ml of saturated $NaHCO_3$, and 50 ml of H_2O. Overnight, the ether extract was dried with Na_2SO_4. Ether was removed by destillation, and the dimethylester was distilled under vacuum (147–149 °C, at 1,5 mm Hg). The carbon content of the dimethyl ester was 67.66% (theoretically 67,57%) and the H-content 9.53% (9.92%). The refractive index was $n_d^{20\cdot0} = 1.4517$. The dimethylester was refluxed with excess

hydrazine hydrate and the same procedure as for the monohydrazine ester above was followed. The carbon content of the product was 60.24% (theoretically 59.12%) and the H-content 9.84% (9.92%). Further recrystallization did not improve purity of the compound. The melting point was 134–137° C. Elementair analysis was made by Mr. W. P. COMBÉ.

Acetylated glyceryl-, mannitol-, and sucrose decenylsuccinate: 66 grams of the monoglycerylester of decenylsuccinic acid were gently heated and refluxed for 30 minutes with 100 ml of acetic acid anhydride and 10 grams of Na-acetate. After cooling, the crystals were dried and recrystallized twice from acetic acid anhydride. To prepare the mannitol ester and sucrose ester of decenylsuccinic acid, the amounts of mannitol and sucrose required to esterify one carboxyl group were added to decenylsuccinic acid and gently heated for 3 hours under constant stirring. The syrups obtained were acetylated as the monoglyceryl ester above, and white needlelike crystals were obtained after recrystallization. Probably, the preparation contains a mixture of compounds, because of the many hydroxylgroups of mannitol and sucrose which may react with the carboxyl group. No further separation of the mixture has been tried. Especially the acetylated mono-esters of mannitol and sucrose were very insoluble in water and only a few experiments on growth retardation were conducted. The melting points for the acetylated monoglyceryl ester, the monomannitol ester, and the monosucrose ester were 145°, 178–182°, and 170–176 °C respectively.

b. Experimental methods.

Water permeability of root cell membranes was measured as water conductivity of a bean root system. The root system of a decapitated bean plant was placed in a potometer with aerated water at 30 °C. A constant suction of 60 cm Hg was applied to the cut end of the stem and the rate of water uptake measured. The meristematic cells of the root tip and of the endodermis cells of the upper root zone account for the main part of the measured resistance to water transport. Damage done to the roots by keeping them at 70 °C for a short time reduces the resistance to water transport to 10% or lower (16). The roots were exposed for 2 hours to a chemical solution and the change in permeability was expressed in % of the original value.

For the study of effects on growth bean seeds var. 'Widusa' were germinated in coarse sand at 25 °C. After a week the seedlings were transferred to Hoaglands's solution, with or without addition of a surface-active chemical. Room temperature varied from 23 to 25 °C, light intensity (400–700mμ) was 30.000 ergs/sec. cm^2 from fluorescent tubes. Initial values of fresh and dry weight, and leaf area were determined. Gain in fresh weight, dry weight, and leaf area are measured for a period of 1 week.

When using *Cucurbita ficifolia*, seeds were placed on wet filter paper at 30 °C in the dark. After 5 days the cotyledons were cut from the emerged seedlings and laid on filter paper moistened with surfactant solution in petri dishes at 30 °C in the light (30.000 ergs/sec. cm^2, 400–700 mμ from fluorescent tubes). The initial fresh weight was measured. After 48 hours the gain in fresh weight was determined.

Experiments on frost resistance were conducted in an insulated box. Plants and flowers were individually marked and placed at random in the box, together with a thermograph. The box was closed and placed in a walk-in refrigerator. Temperature inside the box dropped gradually at a rate of 0.4 – 0.8 °C per hour from +2 °C to –6 °C. In this way, two essential characteristics of a night frost in the open air are simulated: a slow rate of cooling and a very low air circulation, which possibly may postpone formation of ice crystals in some plant cells by supercooling.

4. RESULTS

a. Compounds with different length of the hydrocarbon chain: alkenylsuccinic acids, alkyltrimethylammoniumbromides, alkylimidazolines, and alkylmethylsulfoxides.

Water permeability. Figure 1 shows the effect of different long chain compounds on water permeability of bean roots. In each group the effectiveness

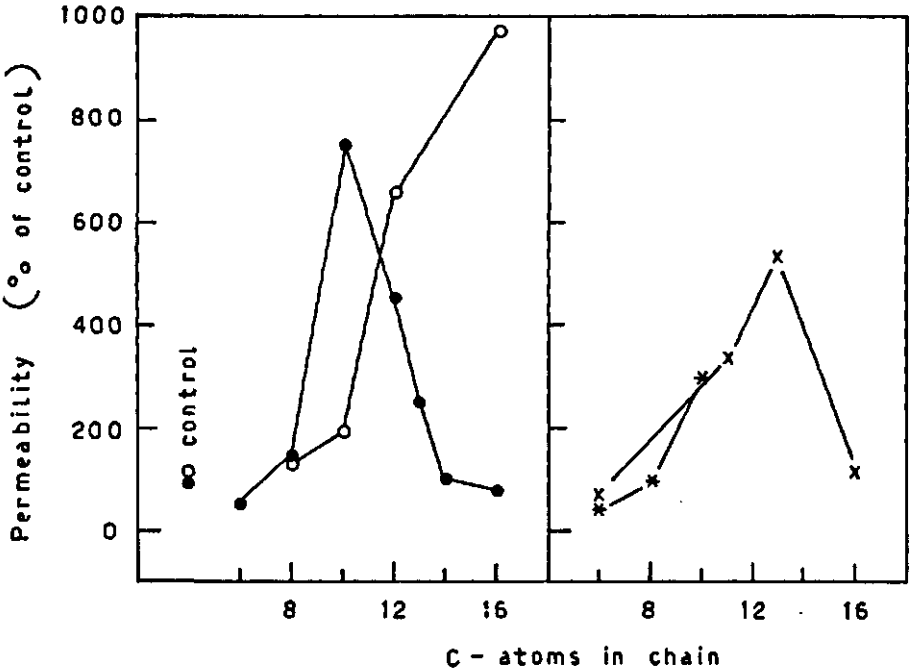

FIG. 1. Effect of surface-active compounds of different hydrocarbon chain length on permeability of bean roots to water. Duration of treatment: 2 hours. ○, 10^{-3}M solutions of 2-alkenylsuccinic acids in aerated water (17), ○10^{-3}M solutionof al kyltrimethylammoniumbromides in water, ×, 10^{-3}M suspensions of alkylimidazolines in aerated water + ethanol (1 %), ★, 10^{-3}M solution of alkylmethylsulfoxides in aerated water + ethanol 1 %, ●, control in water, ○, control in 1 % ethanol.

increases with increasing number of C-atoms in the hydrocarbon chain, up to the point where water solubility of the compound is limiting, e.g. with a solution of undecenylsuccinic acid (and higher) and of tridecylimidazoline. For each group the effect of addition of a CH_2-group to the chain (Q-CH_2) has been calculated and is given in table 1. The value of this quotient varies between 1,6 and 2,1, and is essentially equal for all groups. The experiments suggest that the effectiveness of many more permeability-changing compounds may possibly be enlarged by addition of a hydrocarbon chain. Comparison of chemicals with the same chain length reveals that the succinic acid group is most active, followed by the trimethylammonium group, the imidazoline group, and the methylsulfoxide group respectively.

Growth retardation. All chemicals retarded growth of the bean plant at a certain concentration. A reduction in leaf area was observed, the leaves were darker green and thicker and stem length was reduced. Also a reduction in fresh weight and dry weight was noticed, though the latter was less pronounced. At sufficiently high concentrations a complete cessation of growth occurred and

TABLE 1. The effect of addition of a CH_2-group to the hydrocarbon chain of a surface-active chemical on water permeability of bean root cells. It is expressed as a ratio, Q-CH_2.

Concentration of the surfactant (M)	2×10^{-4}	5×10^{-4}	10^{-3}
alkyltrimethylammoniumbromides	1.6	1.8	1.94
alkenylsuccinic acids			2.1
alkylimidazolines	1.6		1.8
alkylmethylsulfoxides			1.9

damage to the plants became visible. The leaves wilted severely and dried out, the stems bleached and collapsed. The plants looked similar to those exposed to benzene vapor. The plasmamembranes probably are solubilized by excess amounts of these chemicals.

The concentration range in which a certain compound was effective was different for all four groups. It was narrow for the alkyltrimethylammonium-bromides and broad for the alkylmethylsulfoxides (Fig. 2). The concentration for the alkenylsuccinic acids and for the alkylimidazolines were intermediate between those of figure 2. Chain length apparently does not affect the width of the effective concentration range.

From the curves of fig. 2 and its analogues the concentration, at which each compound produces 50% inhibition of the expansion of the leaves has been determined. These values are given in table 2, together with the value of Q-CH_2. Values for gain in fresh weight are similar to those for gain in leaf area but they are about 3 times higher. Essentially, the Q-CH_2-values are similar to those obtained in experiments on water permeability, and evidently the effect of chain length is a phenomenon not restricted to membrane permeability alone.

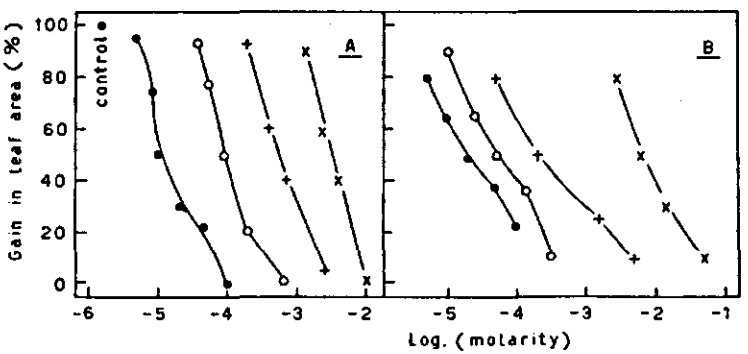

FIG. 2. Effect of surface-active compounds of different hydrocarbon chain length on gain in leaf area of the first pair of leaves of bean plants. A: 1 hour treatment of the roots with alkyltrimethylammoniumbromides with 16(●), 12(○), 10(+), and 8(×) C-atoms in the hydrocarbon chain. B: continuous treatment of the roots in nutrient solution with alkylmethylsulfoxides with 10(●), 8(○), 6(+), and 1(×) C-atoms in the hydrocarbon chain. The initial leaf area varied between 12 and 16 cm^2, the gain in leaf area of the control was 55 cm^2. Duration of the experiment 1 week.

TABLE 2. Effect of long-chain compounds on retardation of growth of the first pair of bean leaves: values of 50% inhibition are given together with the value of Q-CH_2, the increase in effectivenes of a compound, when a CH_2-group is added to the chain.

Compound	50%-value (M)	Q-CH_2	treatment of roots
cetyltrimethylammoniumbromide	10^{-5}		
dodecyltrimethylammoniumbromide	10^{-4}	2.0	1 hour
decyltrimethylammoniumbromide	5×10^{-4}		
octyltrimethylammoniumbromide	3×10^{-3}		
decylmethylsulfoxide	2×10^{-5}		
octylmethylsulfoxide	5×10^{-5}	1.9	continuous
hexylmethylsulfoxide	2×10^{-4}		
dimethylsulfoxide	6×10^{-3}		
tridecenylsuccinic acid	5×10^{-6}		
dodecenylsuccinic acid	8×10^{-5}	1.7	1 hour
decenylsuccinic acid	2×10^{-4}		
hexenylsuccinic acid	2×10^{-3}		

b. Carbonamides of decenylsuccinic acid: decenylsuccinamic acid, decenyl-NN-dimethylsuccinamic acid, decenylsuccinichydrazide, decenyl-NN-dimethylsuccinichydrazide, and decenylsuccinicdihydrazide.

Growth retardation. The effect of decenylsuccinic acid and its monoamides was studied at different pH values (Fig. 3). The effectiveness of the acid was greater at low pH, indicating that the undissociated molecule was responsible for the reduction in growth. At pH 4, the free acid and the dimethylhydrazide were most active. At pH 6,5 the free acid was the least active compound. Addition of an NH_2-group increased activity. The dimethylamide was still more active. Activity of the hydrazide- and the dimethyl hydrazide was again slightly greater than that of the dimethylamide, though the differences were not appreciable. This may have been due to the formation of an internal H-bond between the first N-atom and the O-atom of the other carboxyl group. At pH 8 solutions of the compounds were unstable and they could only be tested as suspensions. All compounds showed a low activity at this pH value, the dimethylhydrazide being most active. The same relationship was found for gain in fresh weight, while smaller differences between the compounds were observed for the gain in dry weight.

The effective concentration range also varied within this series of amides. Decenylsuccinic acid showed the narrowest range, and the dimethylhydrazide the widest one, the curves of the other amides being intermediate. A wide effective concentration range was also characteristic for the dimethylhydrazide of succinic acid itself, B-9 (Fig. 4). The 50%-values of leaf growth retardation are 5×10^{-5}M for B-9 and 500 times less, 10^{-7}M for decenyl-B-9, the dimethylhydrazide of decenylsuccinic acid. The Q-CH_2 value was slightly less than 2.0. The mono-amides were also tested as 10^{-3}M sprays, using 0.1% of a nonionic wetting agent. Sprays of the dimethylamido- and of the NN-dimethylhydrazides were far more active than those of the others. This may be attributed to a rela-

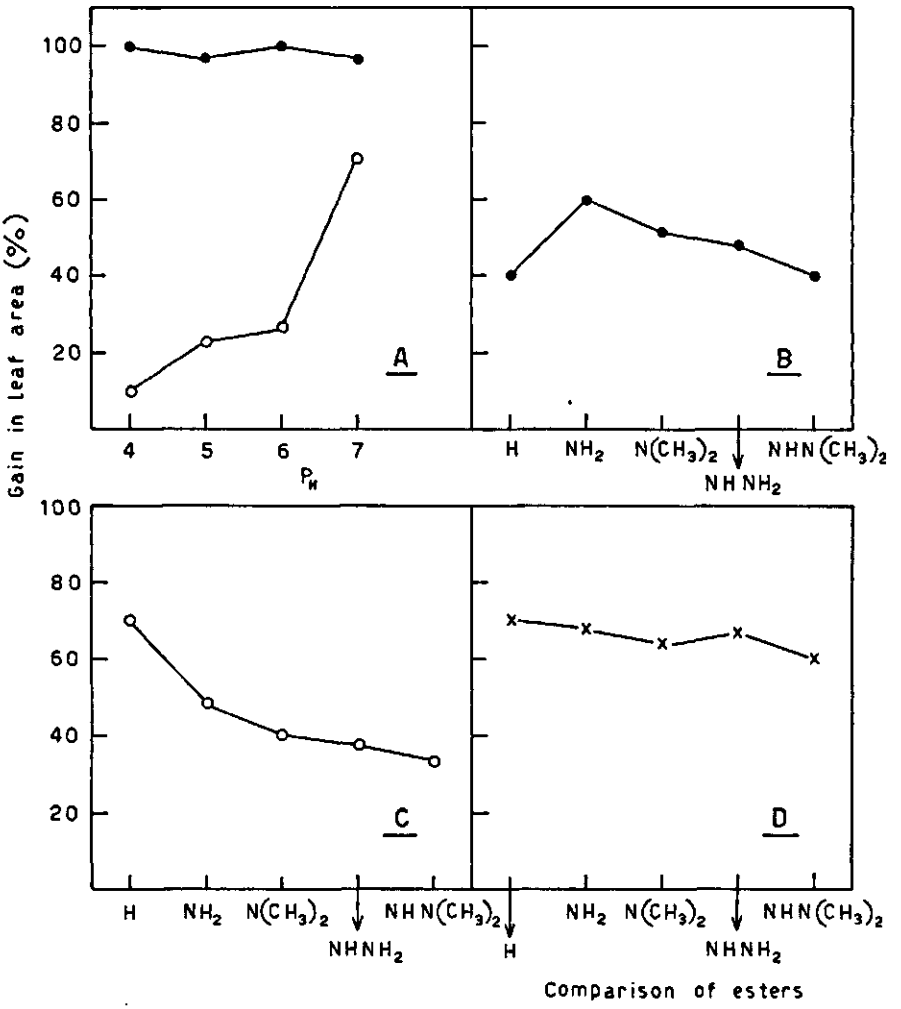

FIG. 3. A: effect of a 1 hour treatment with decenylsuccinic acid (○; 2×10^{-4}M) at different pH on gain in leaf area of the first pair of leaves of bean plants. ●, control. B, C, and D: continuous treatment of the roots in nutrient solution with 10^{-5}M solutions of carbonamides of decenylsuccinic acid at pH 4 (B), 6,5 (C), and 8 (D). H = decenylsuccinic acid, NH_2 = decenylsuccinamic acid, N $(CH_3)_2$ = decenyl-NN-dimethylsuccinamic acid, $NHNH_2$ = decenylsuccinichydrazide, and $NHN(CH_3)_2$ = decenyl-NN-dimethylsuccinichydrazide. The control values for the gain in leaf area are 97% (pH 4), 100% (pH 6,5), and 92% (pH 8). Initial value of leaf area 15 cm^2; gain in leaf area of control 60 cm^2; duration of the experiment 1 week.

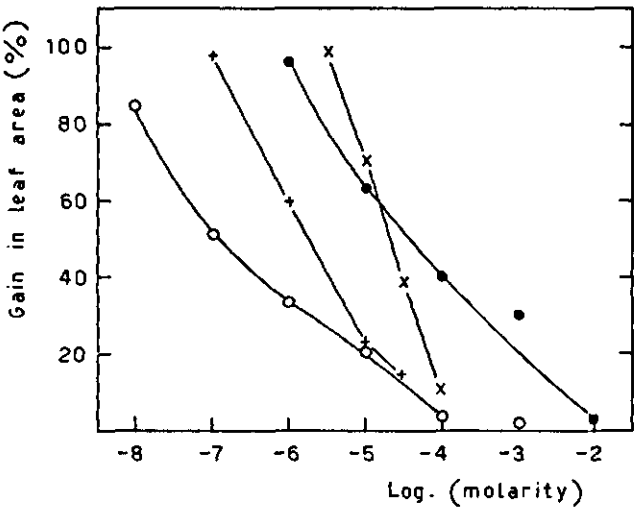

FIG. 4. Effective concentration range of NN-dimethylsuccinichydrazide (●), decenyl-NN-dimethylsuccinichydrazide (○), decenyl-NN-dimethylsuccinamic acid (+), and decenylsuccinic acid (×). The bean roots are continuously exposed to the surface-active compound in the nutrient solution. Further details as in fig. 3.

tively high solubility of these 2 compounds or to a better penetration of the chemicals into the leaf. The effect of a spray with the acid wears off quickly, while a spray with the dimethylhydrazide lasts much longer; a spray with the dimethylamino ester had an intermediate effect. Finally the monohydrazide was compared with the dihydrazide and the results on gain in leaf area in 2 tests are given in fig. 5 at 10^{-5}M concentration. The diamide was much more effective than the mono-amide, even when a comparison was made between equal amounts of hydrazide groups (Fig. 5 B).

Growth stimulation. Gain in fresh weight of excised cotyledons of *Cucurbita ficifolia* in water or nutrient solutions is given in table 3. Treatment with decenylsuccinic acid stimulated gain in fresh weight in the range from 10^{-5} up to 2×10^{-4}M. At higher concentrations no reliable results were obtained due to fungus infections. Gain in dry weight was not affected. Comparison of the mono-amides at 10^{-5}M shows that the stimulatory effect increased in the following sequence: free acid $<-NH_2$ $<-N(CH_3)_2$ $<-NHNH_2$ $<-NHN(CH_3)_2$. This order was the same as that for growth retardation of beans, indicating that an increase in H-bonding capacity induced stronger effects on growth retardation as well as growth stimulation. It is difficult to explain the stimulatory effect of these compounds. Decenylsuccinic acid has been reported to increase photophosphorylation of isolated chloroplasts at very low concentrations (32), possibly by increasing permeability and transport of the phosphate ion into the chloroplast. An increase in permeability of membranes in the young cotyledons may facilitate transport of substances needed for cell growth.

The effect of these compounds depends on the stage of development of the plant. In preliminary tests, stem growth of young *Cucurbita* plants was retarded by sprays of decenylsuccinic acid and its dimethylhydrazide, the latter being more effective.

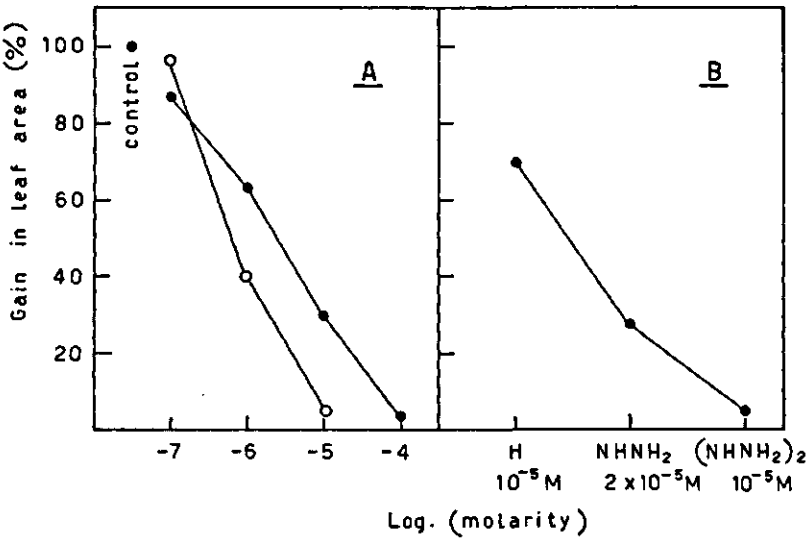

FIG. 5. Comparison of the effect of decenylsuccinichydrazide (A: •, B: $NHNH_2$), of decenylsuccinicdihydrazide (A: ○, B: $(NHNH_2)_2$), and of decenylsuccinic acid (B: H) on growth retardation. Further details as in fig. 3 and 4.

Resistance to freezing. Strawberry flowers were exposed to a period of cooling for 4 hours, then remained for 6 hours at −4 °C, followed by a period of 3 hours of slowly rising temperature. Only 5–12% of untreated flowers survived this treatment. Spraying the flowers with 10^{-3}M solution of decenylsuccinic acid or its mono-amides (to the dripping point) gave no protection in tests 15 hours later. Two or three days after treatment, however, some protection was observed (Table 4). Decenylsuccinic and decenylsuccinamic acid were com-

TABLE 3. Increase of fresh weight (mg) of excised cotyledons of *Cucurbita ficifolia* for 24 hours in water or solution. Initial fresh weight 103 mg. Data represent an average of 20 cotyledons.

Compound	Concentration (M)	Increase in fresh weight (g)	Comparison (%)
water		16	100
decenylsuccinic acid	10^{-5}	19	119
decenylsuccinamic acid	10^{-5}	19.5	122
decenyl-NN-dimethylsuccinamic acid	10^{-5}	31.5	196
decenylsuccinichydrazide	10^{-5}	29	182
decenyl-NN-dimethylsuccinichydrazide	10^{-5}	46	290
decenylsuccinic acid	10^{-5}	20.5	129
decenylsuccinic acid	2×10^{-5}	22	138
decenylsuccinic acid	4×10^{-5}	23	142
decenylsuccinic acid	10^{-4}	23	142
decenylsuccinic acid	2×10^{-4}	24.5	154

TABLE 4. Protection of strawberry flowers against freezing damage by sprays of carbonamides of decenylsuccinic acid at 10^{-3}M concentration. The duration of the freezing period was 6 hours at −4 °C.

Compound	Number of flowers treated	Survival (%)
Water	100	8
decenylsuccinic acid	100	10
decenylsuccinamic acid	100	12
decenyl-NN-dimethylsuccinamic acid	100	30
decenylsuccinichydrazide	40	32
decenyl-NN-dimethylsuccinichydrazide	100	40

pletely ineffective, while an increase in survival is noted among flowers treated with decenyldimethylsuccinamic acid, decenylsuccinichydrazide, and decenyl-NN-dimethylsuccinichydrazide respectively.

The styles of flowers of apple and peach were protected against spring frost only a few hours after treatment with decenylsuccinic acid (1,8). Evidently, a longer time is required for protection of the much thicker developing 'fruit' inside the strawberry flower. An explanation of the protection of the strawberry flowers by the mentioned three mono-amides could be a relatively high rate of penetration. Considering the data on the effect of pH on retardation of bean leaf growth (Fig. 3), it may be suggested that the acidity of the plasma membrane determines the effectiveness of the free acid and its amides. The free acid is highly active at low pH, so that it will preferably protect cell membranes with a high number of acidic groups. The cell membrane of fruit flowers may well be an example. They are protected against frost by a spray with 10^{-3}M decenylsuccinic acid (18,25). At pH 6.5 the free acid is far less active than the three mono-amides mentioned above. Possibly, protection of the cell membranes of strawberry flowers by these amides may be attributed to a lower number of acidic groups in the membrane.

c. Acetylated compounds: glycerol mono-, di-, and tri-acetate, glucosepentaacetate, sucroseoctaacetate and acetylated monoesters (glyceryl-, mannitol-, and sucrose-) of decenylsuccinic acid.

TABLE 5. Effect of glucosepentaacetate and of sucroseoctaacetate on water permeability of bean roots cells, measured as water uptake under a suction of 60 cm Hg. The data are given as % of initial value and represent an average of 5 plants.

Compound	Concentration (M)	Time after application (hours)			
		0	2	5	8
water + ethanol (1%)		100	105	89	83
glucosepentaacetate	10^{-3}	100	68	120	140
glucosepentaacetate	5×10^{-3}	100	87	125	144
sucroseoctaacetate	10^{-3}	100	64	140	191
sucroseoctaacetate	5×10^{-3}	100	140	187	268

Water permeability. Treatment of bean roots with a 1% ethanol solution of glucosepentaacetate or sucroseoctaacetate (ethanol alone in this concentration is inactive) did slightly increase water conductivity, though only after a relatively long time (Table 5). Also the effective concentration was high. For a comparison of the effect of the number of acetyl groups, a more sensitive test, electrolyte permeability, was used.

Electrolyte permeability. An example of the time course of electrolyte permeability of bean roots is given in fig. 6. Roots were transferred from HOAGLAND solution to distilled water or to a solution of a specific compound, and an increase in electrical conductivity of the solution surrounding the roots with time was recorded. This increase in a solution of glucosepentaacetate was much faster thans was the increase in water. No differences between the control solution and the glucose solution were observed. This also applied to sucrose or glycerol solutions. Fig. 7 represents a number of curves of electrolyte permeability of solutions of acetylated glycerol and sugars. The values are corrected for leakage of electrolytes from control roots in distilled water. At equal molar solutions, the effectiveness of the compounds increased with the number of acetyl groups from 1 up to 8. Comparison of solutions with equal amounts of acetyl groups showed that the increase in effectiveness was less than proportional to the number of acetyl groups in the solution (Fig. 7B).

Growth retardation of beans. Acetylated glycerol and acetylated

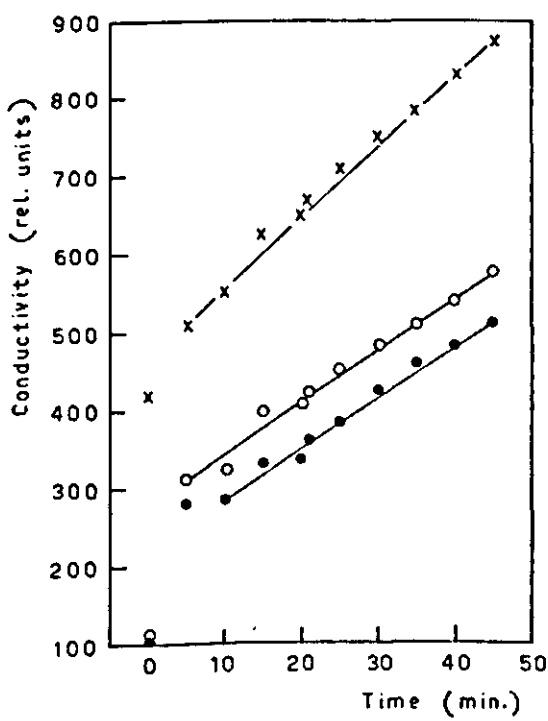

FIG. 6. Time course of electrolyte permeability of bean roots placed in distilled water (●), 4×10^{-4}M glucose-solution (○), or 4×10^{-4}M solution of glucosepentaacetate (×). Five bean plants are placed in 500 ml of water or solution. Electrolyte permeability is measured as electrical conductivity of the root solution. 250 units on the vertical scale correspond to $10^4\ \Omega$.

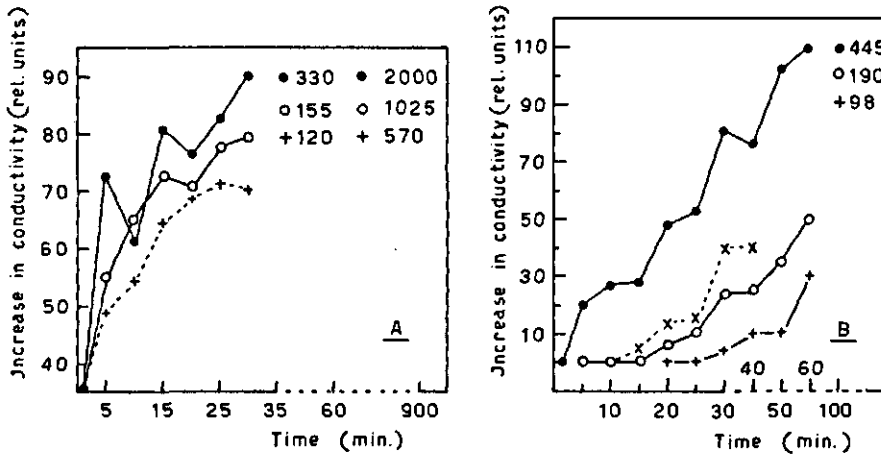

FIG. 7. Time course of electrolyte permeability of bean roots placed in water or in solution (10 bean plants in 500 ml). The curves are corrected for permeability from control plants in water, glycerol, glucose, or sucrose. A: 10^{-3}M solution of glyceroltriacetate (•), glyceroldiacetate (○), and glycerolmonoacetate (+). B: +, 4×10^{-3}M solution of glyceroltriacetate in 1% ethanol, •, $5/3 \times 4 \times 10^{-3}$M solution of glyceroltriacetate in 1% ethanol, ○, 4×10^{-3}M solution of glucosepentaacetate in 1% ethanol, ×, 4×10^{-3}M suspension of sucroseoctaacetate in 1% ethanol.

sugars had a wide effective range (Fig. 8A). The number of acetyl groups essentially had no effect on the width of this range. Effectiveness, increased with the number of acetyl groups in the molecule, roughly 1,8 times for each added acetyl group. Comparison of the compounds at equal amounts of acetyl groups in the solution again showed that the increase in effectiveness was less than proportional to the number of acetyl groups in solution (Fig. 8 B). This may be attributed to the wide effective concentration range of all tested compounds.

Similar results were also obtained when solutions of these chemicals were sprayed to the dripping point on the bean leaves. Sprayed plants generally resumed growth again after one week, indicating that the acetylated compounds are metabolized by the plants.

Fig. 9 shows the effect of a long chain attached to an acetylated compound. The acetylated monoglyceryl ester at 10^{-5}M showed the same growth retardation as did glycerol diacetate at a 200 times higher concentration (2×10^{-3}M). Results with the acetylated mono-mannitol- and mono-sucrose-ester of decenylsuccinic acid were rather disappointing. The compounds could only be tested as suspensions, and at 10^{-5}M concentration no significant differences between the acetylated glyceryl-, and sucroseester were measurable.

Growth retardation of *Cucurbita* cotyledons. No stimulation of gain in fresh weight of excised cotyledons of *Cucurbita ficifolia* was observed with most of the acetylated sugars. Growth was retarded by the acetylated compounds, though this retardation was smaller than the retardation induced by

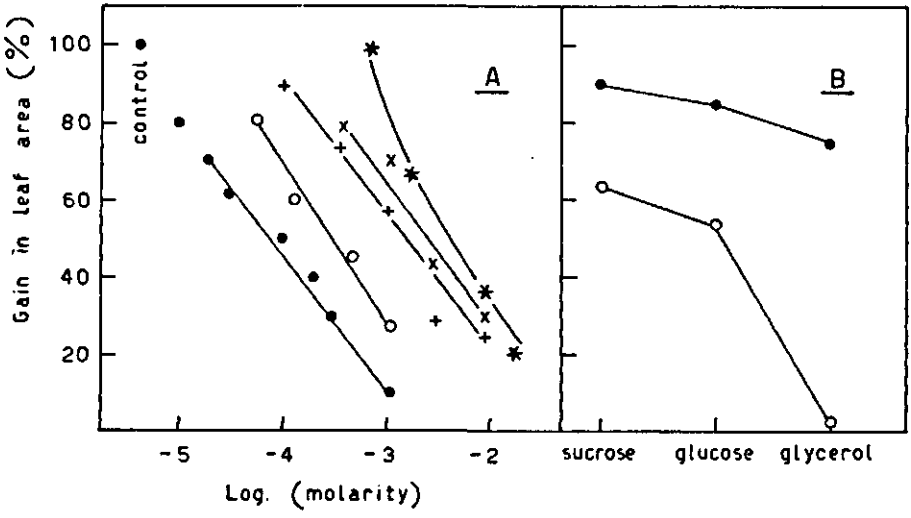

FIG. 8. Effect of acetylated surface-active compounds on gain in leaf area of the first pair of leaves of bean plants. The nutrient solution with the surface-active agents was renewed each day. A: ○, sucroseoctaacetate, ○, glucosepentaacetate, +, glyceroltriacetate, ×, glyceroldiacetate, ★, glycerolmonoacetate. B: ○, comparison at equal amounts of acetyl groups in the solution, of sucrose octaacetate (10^{-3}M), of glucosepentaacetate ($8/5 \times 10^{-3}$M), and of glyceroltriacetate ($8/3 \times 10^{-3}$M). The corresponding date on sucrose, glucose and glycerol (•) at the same concentrations as the acetylated analogues are included. Initial leaf area 12 cm²; gain in leaf area of control 58 cm²; duration of experiment 1 week.

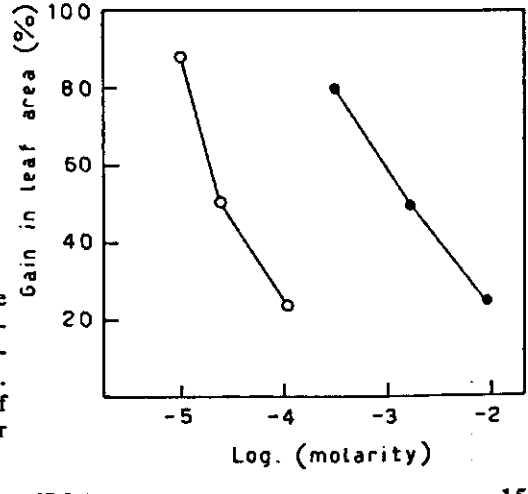

FIG. 9. Comparison of the effective concentration range of glyceroldiacetate (•) and of the acetylated monoglycerylester of decenylsuccinic acid (○). Initial leaf area 18 cm²; gain in leaf area of control 55 cm². For further details see fig. 8.

the corresponding amounts of glycerol and sugars (Table 6). The experiment suggests that at least part of the induced growth retardation by the acetylated compounds is due to deacetylation.

Growth stimulation of potato. Tubers of the variety 'Libertas' were planted May 10, 1966 in the field. The plants were sprayed twice with glyceroltriacetate, sucroseoctaacetate, and the acetylated monoglycerylester of decenylsuccinic acid, viz. on July 6 when they were 30 cm high, and on July 28. At harvest, August 16, the foliage was still green, with a few yellowing leaf edges. The results obtained are summarized in table 7. Tuber yield was not affected by the acetylated monoglyceryl ester of decenylsuccinic acid, while a decrease in

TABLE 6. Increase in fresh weight (mg) of excised cotyledons of *Cucurbita ficifolia* for 48 hours in water or in 10^{-3}M solution. In the last column a comparison is made between the acetylated compound and its corresponding sugar or glycerol. Initial fresh weight 115 mg. Data represent an average of 20 cotyledons.

Compound	Increase in fresh weight (mg)	Comparison (%)	
water	70.2	100	
glycerol	58.5	83.7	100
glyceroltriacetate	61.3	87.6	104.7
glucose	57.1	81.7	100
glucosepentaacetate	69.2	99.0	115.5
sucrose	60.0	85.9	100
sucroseoctaacetate	71.3	101.9	118.5

TABLE 7. Effect of acetylated compounds used as a spray on leaf growth of potato and tuber yield in a field test.

Compound	Fresh weight foliage						
	conc. (M)	g/plant	%	stand. error (σ)	stand. dev. of mean (σñ)	significance (P)(%)	effect of treatment
1. water		730	100	129	37.2		
2. sucroseoctaacetate	2×10^{-3}	613	84.0	105	29.5	$90<P<95$	reduction
3. glyceroltriacetate	10^{-2}	863	118.2	214	61.8	$P=90$	stimulation
4. acetylated monoglyceryl ester of decenylsuccinic acid	10^{-3}	609	83.4	124	35.7	$P<90$	reduction

Compound	Tuber yield				
	g/plant	%	standard error (σ)	standard deviation of mean (σñ)	significance (P) (%)	effect of treatment
1.	513	100	79.5	22.1		
2.	463	90.3	82.8	23.0	$P = 90$	reduction
3.	651	126.9	132	38.2	$97.5 < P < 99$	stimulation
4.	502	98.0	91.3	26.4		none

foliage was noted. Sucrose octaacetate reduced foliage (fresh weight) as well as tuber yield. Part of this reduction may have been due to the ethanol needed to dissolve the chemical. Treatment with glyceroltriacetate significantly stimulated foliage as well as tuber yield (viz. 18.2% and 26.9% respectively). No differences in dry weight percentage of treated and untreated tubers were found. The statistical variation of foliage and tuber yield of the control plants was similar to that of plants treated with sucroseoctaacetate or with the acetylated monoglyceryl ester. The growth stimulation with glyceroltriacetate showed a larger statistical variation. Therefore, it is difficult to judge, whether the increase in tuber yield was entirely due to increased foliage. Data of the individual plants suggested that treated plants had higher yields of tubers for the same amount of foliage than control plants (Fig. 10). An increase in statistical variation of yield when a growth regulator was applied, has often been observed,

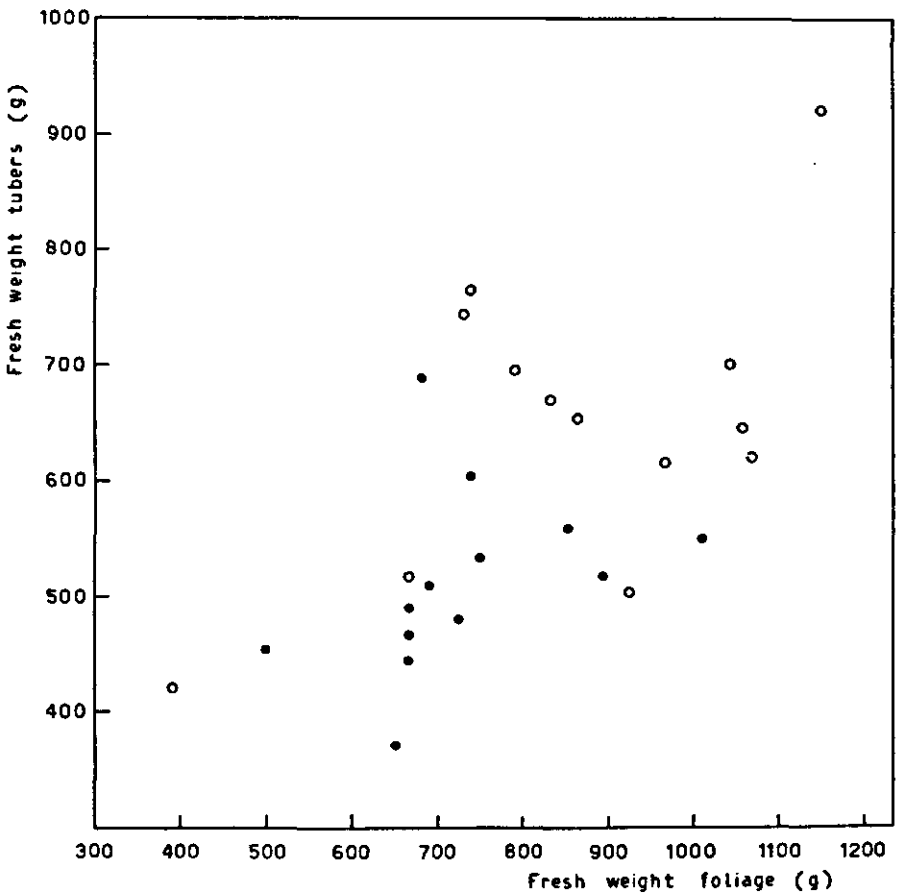

FIG. 10. Relation between fresh weight of foliage and fresh weight of tubers of potato plants in the field. ●, untreated plants ○, treated plants, sprayed twice with a 10^{-3}M solution of glyceroltriacetate to the dripping point.

e.g. in grapes treated with CCC (8). For this reason, a carefull statistical analysis is always necessary to judge reliability of a yield increasing treatment.

d. Fluorinated compounds: 1,5-difluoro-2,4-dinitribenzene and 1-fluoro-2,4-dinitrobenzene.

Water permeability. A few tests demonstrated that water permeability of bean roots was increased by a treatment with the two fluoro-compounds (Table 8). 1-monofluoro-2,4-dinitrobenzene was more effective than 1,5-difluoro-2,4,-dinitrobenzene.

Growth retardation. The effective concentration range in bean plants of the difluoro compound was wider than that of the monofluoro compound. Therefore, the monofluoro compound was more effective at a concentration higher than 2×10^{-5}M, while below this concentration the reverse was true.

Resistance to freezing. Bean plants were individually marked and placed at random in an insulated box. Temperature inside the box was lowered gradually to –3 °C and kept constant at that temperature. Plants were exposed to freezing periods of 4, 8, 24 and 48 hours. The results are given in table 9. 1-fluoro-2,4-dinitrobenzene was ineffective to protect plants against freezing, while 1,5-difluoro-2,4-dinitrobenzene induced resistance to frost. Plants survived 4 and 8 hours at –3 °C without any damage, while longer periods of frost caused

TABLE 8. Effect of 1,5-difluoro-2,4-dinitrobenzene and of 1-fluoro-2,4-dinitrobenzene on water permeability of root cell membrane measured as water uptake under a suction of 60 cm Hg. The data are given in % of the initial value and represent an average of 5 plants.

Compound	Concentration	Time after application (hours)		
		0	2	3
water		100	95	89
1-fluoro-2,4-dinitrobenzene	5×10^{-4}	100	200	320
1-fluoro-2,4-dinitrobenzene	10^{-3}	100	260	500
1,5-difluoro-2,4-dinitrobenzene	5×10^{-4}	100	120	200
1,5-difluoro-2,4-dinitrobenzene	10^{-3}	100	130	360

TABLE 9. Protection of bean plants against freezing damage by treatment of the roots for 1 week with fluoro-compounds or other surface-active chemicals. Freezing temperature –3 °C. Each treatment consists of 25 plants. Survival is expressed as percentage.

Compound	Concentration (M)	Freezing period (hours)			
		4	8	24	48
water		16	0	0	0
1-fluoro-2,4-dinitrobenzene	10^{-5}	8	0	0	0
1,5-difluoro-2,4-dinitrobenzene	10^{-5}	100	52	24	12
decenylsuccinic acid	10^{-5}	96	8	0	0
decenyl-NN-dimethylsuccinichydrazide	10^{-5}	92	12	0	0
NN-dimethylsuccinichydrazide	10^{-4}	88	4	0	0
octylmethylsulfoxide	10^{-5}	92	0	0	0

severe damage to the leaves, and only the stem and petioles survived. Later, new shoots developed.

A few tests of other chemicals are included in table 9. A slight increase in frost resistance was noted with compounds of a relatively large effective concentration range. Cetyltrimethylammoniumbromide, which is characterized by a narrow effective concentration range, was ineffective. Thus far, the effect of this protection has been very small, and more experimentation is needed before these tests result in a completely reliable protection of bean plants against freezing damage in spring.

5. Discussion

To be useful as a growth regulator, a surface-active type of chemical must fulfill a number of requirements. To induce a growth retardation that affects the ultimate size of the mature plant the compound should not be metabolized too quickly, nor too slowly. The effect of a treatment with decenylsuccinic acid on beans wears off quickly under favourable condition of respiration (19) and use of this compound to reduce size of bean plants would not be practical. The retarding effect of B-9 (NN-dimethylsuccinichydrazide) on the other hand often lasts too long, as demonstrated in apples (10). Vegetative shoot growth is effectively reduced, but, unfortunately, fruit growth is also reduced. Decenyl-NN-dimethylsuccinichydrazide (decenyl-B-9) also shows a long lasting effect on beans. Decenyl-NN-dimethylsuccinamic acid is intermediate between the free acid and the above compound. This suggests that the duration of the effectiveness can be regulated by the number of N-atoms of the ester. For some applications such as the temporary protection of the styles of fruit flowers against night frost, the compound should be metabolized in a short period to prevent long term effects on growth. In this respect decenylsuccinic acid shows promise in laboratory tests (18) as well as orchard application (25)[1].

A second requirement is a wide effective concentration range of the compound. This is observed for B-9 (Fig. 4), CCC (7), decenyl-B-9 (Fig. 4), the alkylmethylsulfoxides (Fig. 2 B), and the acetylated compounds (Fig. 8 A). Because of the narrower concentration range the alkyltrimethylammoniumbromides and the alkenylsuccinic acids are less attractive for growth retardation of beans. Generally, reports of injury caused by growth retardants are concerned with compounds with a relatively narrow effective concentration range (24). Comparison of the carbonamides of decenylsuccinic acid (Fig. 4) shows that the width of the effective concentration range depends on the number of N-atoms of the esterified group, but is independent of the length of an attached hydrocarbon chain. The number of acetyl groups of acetylated compounds neither affects it. However, the effective concentration ranges of bifunctional compounds such as 1,5-difluoro-2,4-dinitrobenzene and of decenylsuccinic-hydrazide respectively are wider and narrower then those of the unifunctional analogues.

[1] See also: Hilborn, M. T. (1966), Maine Farm Res. 14 (3).

To my opinion, the effect of a long hydrocarbon chain promises very interesting possibilities for growth regulators. Growth of several plant species responds only to a very high dosage of a small molecule surfactant, as e.g. B-9 and CCC. A similar effect may be expected by much lower concentrations of the long chain analogues, since in the described tests each C-atom of the chain increases about two times the effectiveness of a compound. Only a few small molecule growth regulators are known. They are all characterized by higly polar groups: 2-chloroethyltrimethylammoniumchloride (CCC), NN-dimethylsuccinichydrazide (B-9), hydroxyethylhydrazine (7). Succinic acid, succinamic acid, and NN-dimethylsuccinamic acid are ineffective. The carbonamides of decenylsuccinic acid and the free acid itself demonstrate that many more chemical groups become functional in growth regulation when a long hydrocarbon chain is attached to the compound. Among the long chain compounds tested, decenyl-NN-dimethylsuccinamic acid shows the best combination of properties as regards effectiveness, high water solubility, persistance inside the plant, effective concentration range, and effective range of pH.

Several long chain compounds retard the growth of beans. The same compounds may stimulate growth of the young cotyledons of *Cucurbita* (Table 3). STOWE (35) reports growth stimulation of pea stems by methyl- and ethyl esters of regular fatty acids, by triglycerides, and by a few monoglycerides (e.g. monolein). Recently, a growth stimulating monoglyceride has been isolated from cucumber seedlings (29). Acetylated glycerol retards growth of bean seedlings and of cotyledons of *Cucurbita*. Because of the effect of the hydrocarbon chain one may expect that acetylated monolein will retard growth at a very low concentration (see also the retarding effect of the acetylated monoglycerylester of decenylsuccinic acid, fig. 9). Though speculative, biochemical regulation of growth of seedlings by acetylation and deacetylation of monoglycerides is certainly a possibility, and a search for such a mechanism seems promising.

One cannot predict if a certain long chain compound will stimulate or retard growth of a certain plant species or plant part. Studying compounds of the structure $CH_2X\text{-}CH_2.N^+(CH_3)_3$. TOLBERT (37, 38) found that they were active as growth retardants when X represents an electronattracting group: Cl, Br, J, $=CH_2$, and others. Other compounds with groups of less electronegative character stimulate growth: glycerol triacetate (acetyl groups) on potatoes (Table 7), and monolein (hydroxyl groups) on pea stem growth (35).

In the tests on potatoes in the field as described here, glycerol triacetate (10^{-2}M) stimulated growth of foliage, while sucrose-octacetate (2×10^{-3}M) and the acetylated monoglycerylester of decenylsuccinic acid (10^{-3}M) reduced foliage. Possibly, all acetylated compounds used will stimulate tuber yield of potato plants at relatively low concentrations, and retard growth at higher concentrations. Tests of these compounds at different concentrations are required to clarify this point. Another interesting observation is the increased statistical variation of tuber yield and foliage of potato plants sprayed with 10^{-2}M glyceroltriacetate (Fig. 10, Table 7). Timing of the treatment may be very critical, and the effect may only be observed when the plants are in a specific

stage of development. More likely, however, the genetical variation of potatoes to this chemical is large. If true this means that selection of potato plants, sensitive to acetylated glycerol should increase tuber yield far above the measured 27% found in this test (See fig. 10). Finally, further field tests will show whether the observed increase in potato yield continuous until harvest time and/or if treatment allowes an earlier harvest.

The site of action of plant hormones such as gibberellin is very close to the genes (40). If the hypothesis of crosslinkage of nucleic acid by radiomimetic compounds (11) is correct, many of these surface-active compounds may interfere directly with growth of cells by cross-linking DNA. Another possibility is an inhibition of gibberellin biosynthesis by such compounds. This has been demonstrated by ZEEVAART for CCC (41).

Plants which are damaged or killed by frost, are limp when thawed. The cells have lost their turgor, the plasma membrane is disrupted by frost. Permeability to water and to electrolytes often is correlated with the degree of a low temperature hardiness, e.g. in apple flower buds (2). Interestingly, the compound decenylsuccinic acid increases water- and electrolyte permeability (17, 24). It also gives protection of fruit flowers against freezing (18, 25). Ionic permeability and sensitivity of the plasma membrane to denaturation by freezing temperature possibly are linked via the membrane adenosine triphosphatase. POST and ALBRIGHT (26, 27) present evidence that adenosine triphosphatase activity of erythrocyte membranes is part of a system for transport of sodium and potassium across the cell membrane. SKOU (33, 34) finds a similar relationship for the adenosine triphosphatase activity of nerve cell membranes. FISHER and HODGES (13) report on an ion sensitive adenosine triphosphatase from oat roots. RACKER (28) observed that adenosine triphosphatase from beaf heart mitochondria is cold labile under certain conditions. The enzyme is denatured in 15 minutes at 0 °C or lower. Potato adenosine triphosphatase (apyrase, Sigma) also is denatured at low temperature in a few minutes (unpublished experiments). The enzyme solution is prepared and stored at 0 °C in 0.005 M tris-HCl buffer at pH 7. Then 0.3 ml of cold 0.003 M adenosine triphosphate is added to 1 ml of enzyme solution and kept at 0 °C for 5 minutes. Subsequent warming to 30 °C reveals that the enzyme has been denatured in the cold. I, therefore, suggest that the membrane adenosine triphosphatase is sensitive to denaturation by frost. Surface-active agents as decenylsuccinic acid may change the configuration of the enzyme complex. This may result in a change of the ion and water transport across the membrane and in some cases in a protection against denaturation of the sensitive groups of the molecular complex. In a few preliminary tests, however, I have not been able to give protection of potato adenosine triphosphatase against cold by any of the surface-active compounds mentioned in this study. Therefore a carefull investigation of the environmental conditions inducing cold lability and ion sensitivity of this enzyme is planned. As regards the protection of plants against frost, field tests of decenylsuccinic acid and its carbonamides on open fruit flowers and on flower buds will have to show if the results obtained under laboratory conditions (Table 4) have prac-

tical value. PHILLIPS tests of decenylsuccinic acid on plum flowers in the orchard (25) is a first field demonstration that these flowers can be effectively protected against night frost.

6. SUMMARY

The effect of 2-alkenylsuccinic acids, alkyltrimethylammoniumbromides, alkylimidazolines, and alkylmethylsulfoxides was tested on water permeability of bean roots and retardation of growth of young bean plants. In each group the effectiveness increased with increasing number of C-atoms in the hydrocarbon chain, viz. roughly two times upon addition of a CH_2-group to this chain. The effective concentration range was narrow for the alkyltrimethylammoniumbromides and broad for the alkylmethylsulfoxides. Chain length did not effect the width of it. The effect of decenylsuccinic acid and of a few of its mono-amides (amide-, dimethylamide-, hydrazide-, dimethylhydrazide-) on growth retardation of beans depended on pH. At pH 4 the free acid was the most active compound. At pH 6.5 the effectiveness of the compounds increased in the above sequence. The same order was observed for stimulation of growth of excised cotyledons of *Cucurbita ficifolia* and for induction of frost resistance in strawberry flowers. Survival of the flowers was respectively 8% (control), 10% (decenylsuccinic acid), 30% (decenyl-NN-dimethylsuccinamic acid), and 40% (decenyl-NN-dimethylsuccinichydrazide). When bean roots were exposed to acetylated glycerol, glucosepentaacetate, or sucrose octaacetate, water and electrolyte permeability of the roots was increased. Effectiveness increased with the number of acetyl groups in the molecule. Growth of beans and of excised *Cucurbita* cotyledons was retarded by the acetylated compounds. In a field test two sprays with 10^{-2}M glyceroltriacetate on potato plants increased fresh weight of foliage 19% and tuber yield 27%. 1,5-Difluoro-2,4-dinitrobenzene gave protection to young bean plants against a freezing period of 8 hours at –3 °C; 1-fluoro-2,4-dinitrobenzene was ineffective.

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