EFFECTS OF DOWCO 242 ON PLANT GROWTH, GA_3 CONTENT AND PEROXIDASE ACTIVITY IN SOYBEAN PLANTS

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Abstract: Dowco 242 (D-242) is a new growth regulator synthesized by the Dow Chemical Company, U. S. A.. It is a derivative of a quaternary ammonium compound. The extent of the inhibitory effects of D-242 on plant growth are dependent upon: (1) Stage of plant growth—the retardative effect of this chemical on stem growth decreases with the age of the plant. (2) Tissue of plant—stem tissues and root tissues show different responses to treatment by D-242. Root growth, unlike the stem elongation, was not reduced by the chemical. Except for initial leaf expansion, the leaf number as well as the leaf area was not altered by treatment with D-242. (3) Concentration of D-242 used—the higher the concentration of D-242 utilized, the more the growth of the stem was inhibited, and a longer time was needed to reach its maximal growth.

D-242 caused the reduction of stem growth because of the declination of GA_3 content in plant. There is a conflict in the relationship between the GA_3 content and peroxidase activity on plant growth. It seems that the peroxidase, which causes the oxidative destruction of IAA, may be enhanced or de-repressed by decreases of GA_3 content in plant. Therefore, it may be concluded that the reason D-242 retards the stem growth of plants is due to the decrease of GA_3 and IAA content in plants.

INTRODUCTION

Since 1949, the utilization of artificial growth retardants, such as Amo-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methylchloride), CCC (2-chloroethyl trimethylammonium chloride), phosphon D (2, 4-dichlorobenzyl tributyl phosphonium chloride). and B-9 (N-dimethylamino-succinamic acid) on plants have been reported[2] It has been demonstrated that if plants are treated with the proper concentration of any of these retardants, the plant does not show any visible external malformation except that the stem becomes shorter[2]. Thus plants, which have been treated with any of these retardants at a suitable concentration, may have more resistance to high wind velocity. But plants show different sensitivity to retardants at different stages of their growth. In 1962, Stuart first demonstrated that the application of growth retardants, phosphon D, CCC, and B-9 caused the suppression of vegetative growth but the promotion of the initiation of floral buds in *Rhododendron* (cited in Cathey, 1964).

Dowco 242 (Tetraisopentyl ammonium bromide), which was synthesized by The DOW Chemical Company, U. S. A., and abbreviated as D-242, is a new synthetic growth retardant. It is a derivative of a quaternary ammonium compound. Its function, unlike the synthetic retardants mentioned above, has not been thoroughly investigated. Therefore, in these experiments, the aim has been to try and ascertain the effects of this new synthetic growth retardant on plant growth, also on gibberellic acid content, and peroxidase activity in plants, so that, the influences of D-242 on plant growth might be clarified.

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MATERIALS AND METHODS

Culture method

Soybean (*Glycine max* L. var. Shih-Shih) seeds were selected and soaked in a 1.5% of NaOCl solution 5 minutes for sterilization, then the seeds were removed and rinsed thoroughly with distilled water. After this, the seeds were divided into six parts and sowed in germinating dishes (20×20 cm), and then treated with the following concentration of D-242 in 0, 1, 5, 10, 25 and 50 ppm. The D-242 was dissolved in a nitrogen-free medium[4]. The environmental conditions for seed germination in the growth chamber were: relative humidity-75%; light intensity-6,000 lux; photoperiod-15 hours; day/night temperature-28/25°C. On the third day after the sowing of the seeds, the seedlings were transplanted from the germinating dishes to large plastic containers (25×30 cm), which were filled with nitrogen-free medium without containing any D-242, and inoculated with *Rhizobium japonicum*. The inoculated seedlings growing in the containers were brought to the growth chamber and kept under the same environmental conditions as that used for seed germination, except that the light intensity was increased from 6,000 to 16,000 lux to provide more favorable growth conditions for the soybean plants. During the growth period, the nitrogen-free media was replaced with fresh media once every 3 days, and was continuously aerated.

Preparation of inoculum

The *Rhizobium japonicum* used for inoculating the soybean seedlings was isolated from mature soybean nodules and purified by the conventional procedures. The purified *Rhizobium japonicum* was cultured on agar slants at 30°C[6]. Before the Rhizobia were used for inoculation, they were transferred from the agar slant to freshly prepared liquid medium and put on a shaker for 48 hours[9]. Then 1 ml of this Rhizobial suspension was proportionally added to 1 liter of nitrogen-free medium in which the soybean seedlings were growing.

Extraction and determination of peroxidase

Samples were taken soon after the seeds were treated with D-242 for examination and then the plants were harvested at 3 day intervals until the 21st day after seed treatment. The harvested cotyledons and young leaves of seedlings were discarded, the stem tissue was separated from root system. Three to five grams of stem or root tissue were washed and then homogenized in a William Polytron with a 0.01 M of Tris-ascorbate buffer containing polyvinylpolypyrrolidon (pH 8.0). The homogenate was filtrated through a miracloth. The filtrate was then collected and centrifuged at a 15,000 g for 20 minutes. The supernatant was collected as crude enzyme peroxidase. The procedures used to determine the peroxidase activity followed the method described by Lu[13]. The protein in the enzyme preparation was measured by Lowry method[12]. The specific activity of peroxidase was expressed as ΔOD_{470}/mg protein-min.

Extraction and determination of gibberellic acid

Stem tissues of control sections and of 10 ppm of D-242 treated sections were collected on the 3rd, 9th, and 15th day after the treatment of seeds, respectively. The harvested tissues were weighed and then fixed immediately with 85% cold methanol in a container. In order to prevent the GA_3 in the tissues from oxidation in the air, the tissues in the containers were flushed with nitrogen gas and then sealed. They were kept in a freezer at a temperature of −10°C. The extraction and determination procedures for gibberellic acid from the frozen tissues were similar to those used by Cheng[3].

RESULTS

D-242 acted as a growth modifier for soybean plants, as the pictures of intact plants show (Fig. 1), the chemical treatment had some inhibition on the initial root growth and

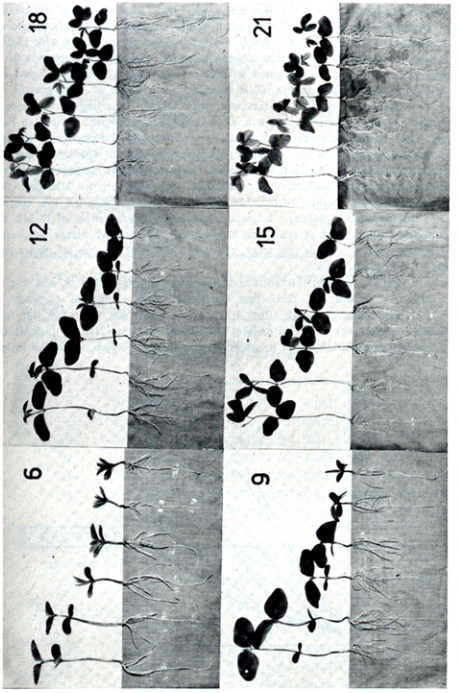

Fig. 1. The effects of D-242 on stem and root growth of soybean plants. The concentration (ppm) of D-242 used to treat the seeds are indicated below the pictures. The number of day shown on each picture was the day on which the plants were harvested and photographed.

development, but at latter stages the root systems of the treated seedlings grew more extensively than the controls. Although the leaf-number and leaf-area of the treated plants was not reduced by the chemical (Table I), the initial leaf expansion was apparently delayed (Fig. 1). However, the chlorophyll content in the leaves was remarkably increased by the treatment (Table I). The fresh weight and dry weight of the plants treated with D-242 were not as heavy as the untreated ones. The shoot/root ratio of treated plants showed a tendency to decline in shoot growth as compared with that of the root (Table II). Based on the growth curves of seedlings shown in Fig. 2, it appears that the rate of stem growth of the treated plants was remarkably reduced by treatment with D-242. The higher the concentration of the chemical used, the more the growth of the stem was suppressed as seen in Fig. 2. This inhibitory effect on stem growth by chemicals was obviously found during the growth period of seedling starting from the 3rd day to 16th day after seed treatment. During this period, the average rate of stem growth of the control plants was 1.65 cm/day, but the rate of growth of those treated with 50 ppm was 0.46 cm/day. However, from the 16th to 21st day, the stem growth of treated seedlings increased from 0.46 cm/day to 0.64 cm/day, but the control decreased from 1.65 cm/day to 0.46 cm/day. It seems that the inhibitory effect of D-242 on stem growth diminished with the age of the plant, consequently, the treated plants recovered their exponential growth phase. But, at this stage, the stem growth of the control plants was not as fast as that of the treated plants.

The elongation curves for the first internode and hypocotyl shown in Fig. 3, and Fig. 4 provide further evidence to support the thesis that the overall stem elongation of plants is repressed by the treatment with chemicals (Fig. 2). Fig. 5 shows the length of the first internode of the plants, which was measured on the last day of harvest (21st day), gradually decreased as the chemical concentration was increased from 1 ppm to 50 ppm, while the length

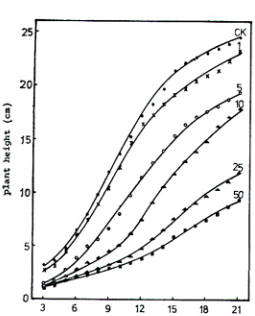

Fig. 2. The effects of different concentration of D-242 on the growth of soybean plants. The number shown at the end of each curve is the concentration (ppm) of D-242 applied to the treated seeds. The plant height was measured beginning the 3rd day after treatment.

Table I. The effects of D-242 on chlorophyll content and the morphological features of soybean plants. These measurements were taken on the 21st day after seed treatment

measurement \ treatment	concentration of D-242 (ppm)					
	0	1	5	10	25	50
chlorophyll content (mg/g fresh weight)	2.59	3.23	3.42	4.26	4.42	4.55
number of nodes	6	6	6	6	6	6
number of expanded leaves	2	2	2	2	2	2
total leaf area (cm^2/plant)	88.93	78.93	88.60	85.18	72.41	76.27
plant height (cm)	24.65	23.30	19.45	17.07	12.05	9.41
length of hypocotyl (cm)	6.06	6.01	4.48	3.34	2.69	2.41
length of 1st internode (cm)	11.55	10.71	8.80	7.01	4.43	3.08
2nd internode	4.10	3.91	4.29	4.50	3.46	2.62
3rd internode	1.85	1.42	1.30	1.09	0.86	0.83
4th internode	0.50	0.39	0.39	0.47	0.31	0.28
5th internode	0.12	0.15	0.12	0.18	0.07	0.10

Table II. Effects of D-242 on the fresh weight, dry weight and shoot/root ratio of soybean plants

A. FRESH WEIGHT

treatment / days	whole plant (g/plant) concentration of D-242 (ppm)						shoot/root ratio concentration of D-242 (ppm)					
	0	1	5	10	25	50	0	1	5	10	25	50
3	0.185	0.168	0.123	0.110	0.094	0.087	1.37	1.08	0.74	0.68	0.77	0.84
6	0.445	0.444	0.284	0.329	0.258	0.228	1.17	1.12	0.80	0.73	0.69	0.74
9	0.755	0.723	0.681	0.635	0.567	0.515	1.80	2.07	1.74	1.18	1.04	0.80
12	1.403	1.622	1.608	1.503	1.134	1.061	2.68	2.21	1.83	1.48	1.19	0.98
15	2.306	2.184	2.134	2.019	1.910	1.854	2.17	2.27	2.26	1.54	1.20	1.19
18	2.466	2.600	2.821	2.584	2.347	2.073	2.36	2.44	2.28	1.72	1.36	1.30
21	3.038	3.140	3.293	3.183	2.583	2.480	2.19	2.27	2.24	2.00	1.45	1.40

B. DRY WEIGHT

treatment / days	whole plant (g/plant) concentration of D-242 (ppm)						shoot/root ratio concentration of D-242 (ppm)					
	0	1	5	10	25	50	0	1	5	10	25	50
3	0.015	0.014	0.012	0.011	0.010	0.009	1.96	1.67	1.39	1.37	1.49	1.59
6	0.037	0.035	0.031	0.030	0.024	0.022	1.89	1.79	1.41	1.40	1.41	1.53
9	0.065	0.065	0.064	0.051	0.049	0.045	2.99	3.72	3.13	1.89	1.98	1.72
12	0.148	0.172	0.149	0.136	0.102	0.095	5.47	4.94	3.67	2.92	2.49	2.06
15	0.257	0.255	0.229	0.199	0.183	0.157	5.01	4.81	4.44	3.12	2.39	3.01
18	0.325	0.318	0.312	0.312	0.209	0.176	4.84	4.76	4.39	3.27	2.28	2.48
21	0.427	0.443	0.440	0.380	0.253	0.222	4.14	4.41	3.37	3.47	2.20	2.36

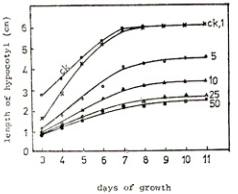

Fig. 3. The effect of D-242 on the hypocotyl elongation of soybean seedlings. The number indicated at the end of each curve is the concentration (ppm) of D-242 used.

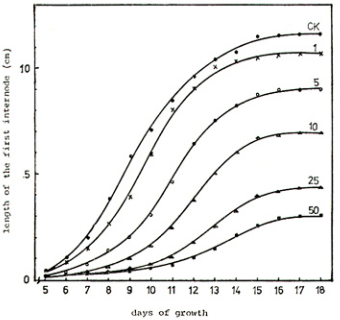

Fig. 4. The effects of D-242 on the elongation of the first internode. The number shown at the end of each curve is the concentration (ppm) of D-242 used in seed treatment.

of the hypocotyl had significantly been reduced by chemical concentrations ranging from 1 ppm to 25 ppm. The length of the hypocotyl, unlike the first internode, was not reduced further by increase of D-242 concentration from 25 ppm to 50 ppm. The average rate of elongation of the first internode of the untreated plants measured from the 3rd to 15th day after seed treatment was 1.53 cm/day, but those treated with the chemical at a concentration of 10 ppm and 50 ppm was 0.86 cm/day and 0.37 cm/day, respectively (Fig. 4). It seems that a longer time (days) is needed to resume effective elongation of the first internode if the plants are treated with a higher concentration of D-242. The plants treated with a concentration of 25 ppm and 50 ppm of D-242, showed that the length of their first internodes seem to

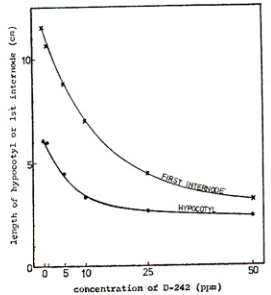

Fig. 5. The effects of the concentrations of D-242 on the length of hypocotyl and of the first internode of soybean plants. Both the length of hypocotyl and of the first internode were measured on the 21st day after seed treatment.

remain unchanged until after 10th day of treatment (Fig. 4). But the control plants and those treated with a low concentration (1 ppm of D-242) had already reached their exponential elongation phase on the 9th day after seed treatment. However, no matter what concentration of the chemical was used, treated plants like the control ones, had stopped the elongation of their first internode on the 17th day after the beginning of the experiment (Fig. 4). Besides the first internode elongation, the hypocotyl elongation was also retarded by D-242. The average length of the plants treated with a concentration of 10 ppm and 50 ppm of D-242 was 55% and 39% of the control, which were measured on the 9th day after treatment. The length of the hypocotyl of the control plants and those plants treated with 1 ppm of D-242 had reached a maximal level on the 7th day after seed treatment, while those treated higher concentration of D-242 had not yet leveled off until after the 9th day of treatment (Fig. 3). Undoubtly, the chemical treatment not only caused a reduction of hypocotyl elongation but also delayed the hypocotyl elongation in reaching the stationary stage.

It has been known that gibberellin in stems controls plant growth and it had also been reported that peroxidase activity interfered with plant growth[10]. In order to find out if the retardative effects on plant growth caused by D-242 is related to the level of gibberellin and the activity of peroxidase, both GA_3 and peroxidase in the stems of the treated and control plants were extracted and determined. Table III shows there was no significant difference in peroxidase activity in roots of treated and controlled plants. During the earlier growth stage, i. e. during the 3rd-12th day after seed treatment, both root of treated and control plants had nearly the same peroxidase activity (Table III), and no apparent differences in the appearance of root growth between treated and control plants were observed (Fig. 1). Then after the 12th day of seed sowing, peroxidase activity in the roots of all D-242 treated plants was lower than that of control plants, but the root system of treated plants grew much better than that of the control ones. The peroxidase activity in the stem is shown in Table III. The peroxidase activity in control plants was significantly lower than in treated plants, but the shoot of the control plants

Table III. The changes of peroxidase activity in stem and root of soybean plants during the growth period

A. Peroxidase activity* in stems

treatment \ days after treatment	3	6	9	12	15	18	21
control	26.19	14.75	16.16	18.44	20.05	19.47	19.04
D-242, 1 ppm	26.21	15.75	17.66	17.93	21.43	21.66	22.80
5 ppm	31.96	22.23	19.18	18.16	19.04	18.27	24.06
10 ppm	34.51	24.67	21.02	17.28	16.91	16.93	20.52
25 ppm	34.28	25.97	26.96	26.27	26.34	24.27	27.81
50 ppm	35.77	26.50	29.06	27.17	28.99	27.05	32.84

B. Peroxidase activity* in roots

treatment \ days after treatment	3	6	9	12	15	18	21
control	43.50	75.93	95.23	175.50	163.63	152.49	140.02
D-242, 1 ppm	36.92	77.30	99.74	175.18	156.46	141.29	138.49
5 ppm	35.25	77.87	93.56	173.07	148.17	129.82	153.95
10 ppm	35.13	71.67	96.26	155.34	151.06	145.63	149.11
25 ppm	45.33	77.09	91.00	146.84	162.27	138.68	105.13
50 ppm	48.90	72.64	99.28	152.60	145.13	133.69	120.88

* peroxidase activity: $\triangle OD_{470}$/mg protein-min.

grew much better than did that of treated plants. These results indicate that there is an opposite relationship between the root and stem growth and peroxidase activity, i. e. peroxidase activity may interfer with plant growth. To compare the extractable GA_3 content in the stems of plants treated with 10 ppm of D-242 and that of control ones, Table IV shows the treated plants contained less extractable gibberellin than the control plants. Obviously, the GA_3 content in the stems was reduced by D-242. From the above facts, it may be concluded that root growth and stem growth were interfered by peroxidase activity and GA_3 content. However, the effects of peroxidase activity and GA_3 content on growth may not function independently but be correlated with each other.

DISCUSSION

D-242 like other growth retardants exerts any inhibitory effect on the rate of stem elongation and on early leaf expansion. But it did not exert any negative influence on leaf size, leaf number or on root growth. The level at which the chemicals effected stem elongation and leaf expansion depended upon the concentration used. It was seen that 1 ppm of D-242 was too low to induce any negative modification of plant growth, but 25 ppm or 50 ppm of D-242 caused drastic retardation to plant growth, thus 10 ppm of D-242 is the best concentration to be applied to plants, which will enable stems to grow so as to be able to withstand strong wind velocity, and thus to protect plants from wind damage, since plants treated with this concentration of D-242 were shown to be stronger and stouter.

The effectiveness of the inhibitory influences of D-242 on plant growth is dependent upon the stage of the growth of the plant. This chemical had a more pronounced inhibitory effect on plant growth at an earlier than at a latter stage. At the earlier growth stage, while the control plants or those treated with a low concentration of the chemical had reached their log phase, those treated with a higher concentration of the chemical still remained at the lag phase. However, at a latter growth stage, i. e. from the 15th day to 21st day after seed treatment, the treated plants had a higher rate of stem elongation than did the control plants. This declination of the chemical effect on plant growth at a latter stage may have been due to the degradation of D-242, which may have been modified by enzymes produced by stem tissues, or due to a loss of chemical effectiveness by endogenous dilution of the D-242 owing to an increase in cell volume or cell number as the plants grew older. At this stage, aging may have been the only factor causing a reduction in the rate of growth of the control plants. However, the overall stem growth of the control plants still remained higher than that of the treated plants.

The D-242 exerted different effects on different parts of plants. The stem elongation, both the internodal elongation and the hypocotyl elongation, was much more sensitive to treatment by the chemical than the root growth was. Consequently, the ratio of shoot to root was decreased.

Undoubtly, there are many kinds of gibberellins in plants and different gibberellins may not have the same regulatory function on plant growth[11]. Since GA_3 was the only kind of gibberellin extracted and determined in these experiments, the effects of GA on plant growth was interpreted as the function of GA_3.

Much of the data presented in these experiments (Fig. 6, Fig. 7, Table III & IV) has

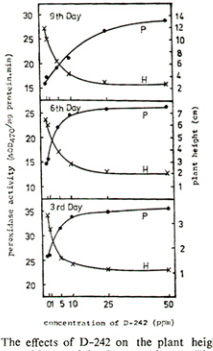

Fig. 6. The effects of D-242 on the plant height and peroxidase activity in stem tissues. The plant height and peroxidase actvity were measured on the 3rd, 6th, and 9th day respectively, after treatment with D-242.
H: plant height. P: peroxidase activity.

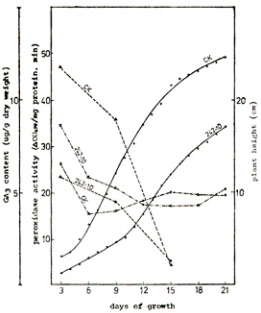

Fig. 7. The effects of D-242 (at 10 ppm) on GA_3 content, peroxidase activity, and plant height of soybean plants. These measurements are indicated as follows: plant height (•—•—•), peroxidase activity (×----×----), and GA_3 content (△---△---△).

Table IV. Comparisons of GA_3 content and peroxidase activity in stem between control plants and treated plants

treatment \ day after treatment / measurement		3	9	15
control	μg GA_3/g fresh wt.	1.076	0.899	0.125
	μg GA_3/g dry wt.	11.759	8.883	1.048
	peroxidase activity*	26.190	16.160	20.049
D-242, 10 ppm	μg GA_3/g fresh wt.	0.809	0.441	0.135
	μg GA_3/g dry wt.	5.869	4.532	1.328
	peroxidase activity*	34.510	21.020	16.915

* peroxidase activity: △ OD_{470}/mg protein-min.
For dry weight measurement, the harvested stems were dried in an oven at temperature 75°C for 48 hours.

demonstrated that there exists a high correlation between rate of stem elongation, GA_3 content, and peroxidase activity. The D-242 treated plants were shorter than the control plants, besides this, these treated plants had a lower GA_3 content and higher peroxidase activity than the control plants. Therefore, it may be concluded that D-242 exerts any direct or indirect effect on GA_3 content and peroxidase activity, which, consequently, resulted in retardation of plant growth. These results are consistent with those found in dwarf plants by Overbeek[18], Halevy[7], Proano[17], Lacoppe[11], Goto[5], and Hoad[8]. It had been previously shown that growth retardants have the ability to inhibit the cell division activity in the subapical meristem[2].

However, we found by examination with a light microscope that D-242 also causes the reduction in cell elongation. Although the mechanism for this inhibitory effect of D-242 on plant growth is not yet understood, we assume that D-242 may block the synthesis or promote the breakdown process of GA_3. Under such conditions due to the decrease of GA_3 content in plants, the peroxidase activity may have been de-repressed or in other words, enhanced, which, in turn, promotes the oxidative destruction of IAA[15,16]. By means of this diminishing of GA_3 and IAA content in the stem, stem elongation was retarded.

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