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The growth-retarding effect of daminozide in *Spirodela oligorrhiza* (Kurz) Hegelm., and its reversal by gibberellin

KRYSTYNA M. JANAS

Laboratory of Plant Growth Substances, University of Łódź,
ul. Banacha 12/16, 90-237 Łódź, Poland

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Abstract

Butanedioic acid mono-(2,2 dimethylhydrazide) (daminozide), in a concentration of 10^{-3} M retarded growth of *Spirodela oligorrhiza* and increased chlorophyll and protein content in the fronds. Gibberellin A_3 and benzylaminopurine partially alleviated the symptoms of daminozide action.

INTRODUCTION

The growth retardant butanedioic acid mono-(2,2 dimethylhydrazide) (daminozide, SADH or B-9) has been reported to retard growth of a wide range of higher plants (Ridell et al., 1962). Kuo and Pharis (1975) have found that daminozide reduced stem elongation in young seedlings of *Cupressus arizonica* Greene. They reported that the endogenous GA content in the seedlings treated with daminozide was at least 11-fold higher than in the control ones.

Williams and Stahly (1970) found that fruits from the 'Red Delicious' apple trees treated with daminozide contained more N--malonyl-D-tryptophan than the untreated control and concluded that this compound may control fruiting and vegetative growth by interfering with auxin biosynthesis. It has been suggested that this compound interferes with the metabolism or regulatory action of either auxin or gibberellin systems or the both (Reed et al., 1965).

2-Chloroethyl (trimethylammonium) chloride (CCC), Alden, and N,N--dimethylmorpholinium chloride (DMMC) retard the growth of duckweed, the effect being reduced by the application of gibberellic acid or benzylaminopurine (Knypl et al., 1976; Knypl, 1977). The aim of this study was to test whether *S. oligorrhiza* is sensitive to daminozide.

MATERIAL AND METHODS

PLANT CULTIVATION

Spirodela oligorrhiza (Kurz) Hegelm. was grown under sterile conditions as described by Bollard (1966) and Knypl et al. (1976). The mineral medium contained 2 mM $(NH_4)_2SO_4$ as the sole nitrogen source and 1% glucose. The medium was sterilized by autoclaving, cooled and supplemented with filter-sterilized (a G-5 filter, Schott Jena) solutions of daminozide, gibberellic acid or N^6-benzylaminopurine (BAP); solid, sterile $CaCO_3$ was added to each flask after autoclaving (Ferguson, Bollard, 1969), to prevent the decrease of pH due to NH_4^+ assimilation. Finally, each flask was inoculated with 3 plantlets (about 10 fronds).

For experiments with growth regulators, 18-ml aliquots of the medium (which was 10% more concentrated than the desired final concentration) were supplemented with 2 ml aliquots of either distilled water (control series) or solutions of growth regulators. The plants were grown under continuous illumination of about 4.6 W cm^{-2} light intensity, produced by "Flora" LF-F and day-light fluorescent tubes (Unitra-Polam, Warsaw) at 25°C.

Growth of the cultures was assessed by counting the number of fronds, and expressed as multiplication rate (MR):

$$MR = (1000 \log F_d - \log F_o) : d$$

where F_o = original number fronds; F_d = number of fronds on day d; d = number of days (Ferguson, Bollard, 1969).

CHEMICAL ANALYSES

The plants of *S. oligorrhiza* after a desired time of cultivation with the growth regulators were transferred to a Büchner funnel, thoroughly washed with tap water followed by distilled water, blotted dry on filter paper and weighed. Chlorophyll was extracted with 80% acetone and determined by Knypl et al. (1976). For the determination of soluble protein, samples of 100 mg fresh weight were homogenized with 1.5 ml of 0.05 M Tris-HCl buffer (pH 7.5) and centrifuged at 3000 X g for 20 min. The supernatant was decanted and the remaining plant material extracted once more with a 1.5 ml aliquots of the buffer. Protein was precipitated with 5% TCA, washed with 80 and 96% ethanol, solubilized in 0.5 N NaOH and determined (Lowry et al., 1951).

RESULTS

Daminozide at low concentrations (10^{-6} to 10^{-4} M) little affected growth of the plants (Fig. 1). In contrast, at higher concentrations of

10^{-3} M and 10^{-2} M the growth rate was reduced, and the plantlets formed large colonies comparising up to 20 overlapping fronds. The progeny fronds showed a tendency to remain attached to the mothers instead of being separated into 3- to 5-frond plantlets as in the case of the control. Roots were shortened to about 1 mm, in comparison with 4 to 5 mm in the control. The multiplication rate (MR) was reduced by about 40% under the influence of 10^{-3} M daminozide (Fig. 2).

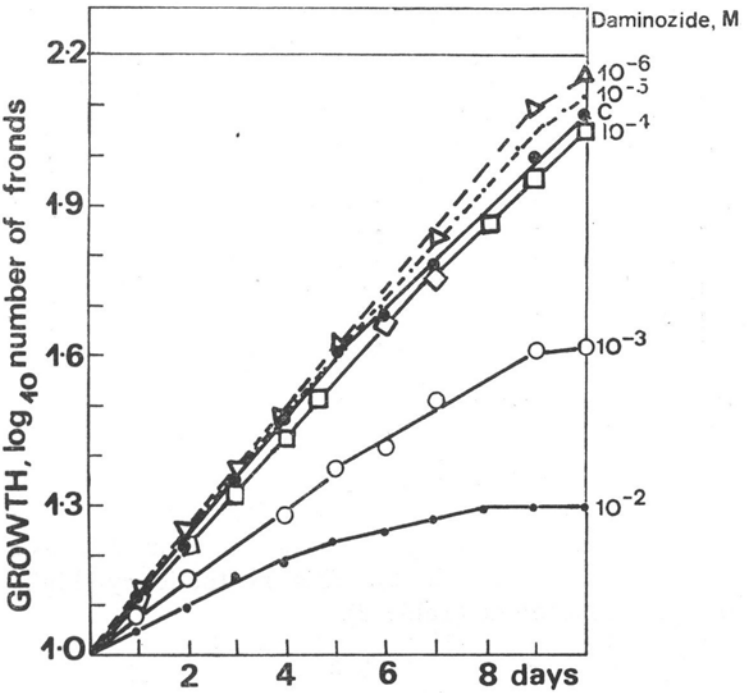

Fig. 1. Kinetics of growth of *S. oligorrhiza* under the influence of daminozide. The plants were grown as described in Material and Methods. Numerals on the right mean molar concentration of daminozide; C — control

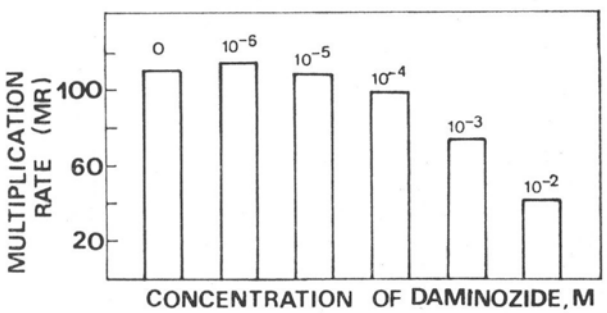

Fig. 2. Effect of daminozide on the growth of *S. oligorrhiza* on the 6th day of growth in continuous illumination.
Numerals above bars mean molar concentration of daminozide; C — control

Daminozide in a range of 10^{-6} to 10^{-3} M concentrations reduced both the chlorophyll and soluble protein contents as analysed after 6 days of growth (Table 1). On the contrary, when *S. oligorrhiza* was grown for 12 days on 10^{-3} M of the compound, the chlorophyll content was higher than in the control plants (Table 1). This effect was still more remarkable in the 10^{-2} M solution of daminozide.

Table 1

Effect of daminozide on chlorophyll and protein contents in *Spirodela oligorrhiza*. Measurements were made after 6 and 12 days of cultivation

Molar concentration of daminozide	Chlorophyll μg g^{-1} fresh weight		Protein mg g^{-1} fresh weight	
	Days of cultivation		Days of cultivation	
	6	12	6	12
0	1950	1620	27	23
10^{-6}	1840	1600	24	23
10^{-5}	1810	1600	24	25
10^{-4}	1700	1460	25	24
10^{-3}	1600	1710	25	25
10^{-2}	2200	1940	27	27

GA_3 (5×10^{-5} M) slightly decreased the chlorophyll content and increased that of protein (Table 2). BAP at 10^{-6} M concentration slightly reduced the MR as measured over the initial 9 days. The plants grown in the presence of BAP became light green; the content of chlorophyll per unit of fresh weight decreased to about 50% as compared with the control plants (Table 2). This synthetic cytokinin slightly reduced the protein content (Table 2).

Table 2

Effect of daminozide, GA_3 and BAP on multiplication rate (MR), chlorophyll and protein contents in *S. oligorrhiza* cultivated for 9 days. Final molar concentrations of the compounds in combined solution were the same as in the solutions containing these compounds alone. Original inoculum: 10 fronds

Treatment M	MR	Chlorophyll μg g^{-1} fresh weight	Protein mg g^{-1} fresh weight
0	110	1850	22.6
GA_3–5×10^{-5}	122	1690	25.5
BAP–10^{-6}	104	900	19.0
Daminozide–10^{-3}	70	1910	24.5
Daminozide +GA_3	114	1830	24.1
Daminozide +BAP	92	1500	22.0

As seen from the data of Table 2 the growth-retarding effect of daminozide at the concentration of 10^{-3} M was completly reversed by GA_3, and markedly reduced by BAP.

Daminozide significantly reduced the inhibitory effect of BAP on the chlorophyll accumulation (Table 2).

DISCUSSION

Responses of *Spirodela oligorrhiza* to daminozide were similar to those described for DMMC and CCC (Knypl et al., 1976). This compound reduced the growth rate and caused the formation of clusters of progeny fronds adhering to the mather plants.

Kuo and Pharis (1975) reported that high levels of daminozide increased the level of endogenous GA-like substances in cyperus seedlings. This increase of the content of endogenous GA-like substances after treatment with daminozide has also been noted in *Brassica oleracea var. italica* (Fontes et al., 1970), potato var. Detskoselsky (Sinelnikova et al., 1972) and other plant species. Since exogenously applied GA_3 could reverse the growth inhibition caused by daminozide in intact coniferous seedlings (Pharis et al., 1967), in *Phaseolus vulgaris* (Bukovac, 1964) and in *Spirodela* (Table 1, 2), it seems justified to postulate that this growth retardant interferes with the biosynthesis of some GA fractions.

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Odwrócenie efektu działania daminozydu przez giberelinę u Spirodela oligorrhiza (Kurz) Hegelm.

Streszczenie

Daminozyd (SADH, B-9) w stężeniu 10^{-3} M hamuje wzrost *Spirodela oligorrhiza* o około 40% oraz podwyższa zawartość chlorofilu i białka. Kwas giberelowy (GA_3) całkowicie odwraca, a benzyloaminopuryna (BAP) zmniejsza objawy hamującego działania daminozydu.