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THE EFFECTS OF SIMAZINE, ATRAZINE AND 2,4-D ON GERMINATION AND EARLY SEEDLING GROWTH OF *ORYZA SATIVA* L.

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Introduction

The effect of triazine herbicides on germination has been a controversial issue for the last 15 years. Gast *et al* (1955, 1956) reported that 2-chloro-4-6 bis (alkylated amino)-s-triazines such as simazine and atrazine do not influence seed germination. Wakoning & Arnason (1958), however, noted that germination of barley was almost completely inhibited by a 24-hour treatment with 200 ppm simazine. Grover (1962) studied the effect of simazine on seed germination of three coniferous species. There was no effect on *Picea pungens* Englem. and *Pinus silvestris* L. but the seeds of *Picea glauca* Moench were seriously affected above 250 ppm. Sasaki & Kozlowski (1968a) and Sasaki *et al* (1968) were unable to find any influence of triazines on seed germination of *Pinus resinosa* Ait.

In conrast to the effect of triazine herbicides, the effects of phenoxyacetic herbicides (e.g. 2, 4-D, 2, 4, 5-T.) have been shown to be inhibitory at moderate concentrations Rojas Garciduenas *et al*, 1962, Kozlowsi & Sasaki, 1968) and stimulatory at low concentration (Husuch & Lou, 1947). The action of phenoxyacetic herbicides is considered to be of phytohormonal nature by most workers. On the other hand, early investigations with the triazines indicated that their action was not phytohormonal in nature (Gast *et al* 1956). More recent investigations have suggested a phytohormonal action (Jordan *et al* 1966; Copping *et al*, 1972).

The aim of this work was to study the action of two triazine herbicides (atrazine and simazine) and one phenoxyacetic herbicide (2,4-D) on germination and early seedling growth of rice (*Oryza sativa* L). The immediate objective was to resolve the controversy of the claimed but clearly disputed, phytohormonal action of the triazine herbicides.

Materials and Methods

Lots of 20 seeds of rice (*Oryza sativa* L. cv. Caloro) were placed on Whatman No. 1 filter paper in 10 cm sterilised Petri dishes which contained 5 ml of an aqueous solution or suspension of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, 50% wettable powder), simazine (2-chloro-4, 6-bis (ethylamino)-s-triazine, 25% wettable powder) and 2-4-D (2-4-dichloro-phenoxyacetic acid) at 5, 50, 100, 250, 500 and 1000 ppm concentrations. All concentrations were based exclusively on the proportion of active ingredients. Distilled water was used as control. Each treatment was

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replicated thrice. The effect of herbicides was tested in light as well as in dark. One set of Petri dishes was kept in a growth chamber maintained at 20± 1°C; light intensity at the top of dishes was 6000 Lux; relative humidity of the chamber varied from 60-75%. The other set of Petri dishes was placed in a dark room maintained at identical temperature and approximately similar humidity conditions as the growth chamber. Small amounts of water were added periodically when it was obvious that Petri dishes were begining to dry out. Seed germination counts were made daily for 16 days. A seed was considered germinated when the coleoptile had attained a length of not less than 1.5 mm (Taylor, 1952). At the end of 16 days, measurements of roots and shoots were made for all the germinated seedlings for each treatment and controls.
Simazine and atrazine were generously presented by Ciba-Geigy, England, to which we owe our sincere thanks.

Results

EFFECT OF HERBICIDES ON SEED GERMINATION

(a) Germination in light: Results of seed germination are presented in Table 1. Greater and rapid germination occurred in light than in dark, which corresponds with the findings of Robert (1961). It appears that simazine at low concentrations. (5,50,100 ppm) accelerated germination initially. At 5 ppm simazine induced a slight increase in percentage germination. Nevertheless at higher concentrations it delayed germination, but the final germination percentage remained unaffected. However, atrazine delayed germination at all the concentrations leaving the final percentage almost unaffected. In contrast to triazines, 2-4-D impeded early germination at 5 and 50 ppm and delayed it at higher dosages. Furthermore, it stimulated germination percentage at 5 and 50 ppm and drastically reduced it at higher concentrations.

(b) Seed germination in dark: Simazine at all doses delayed germination in dark. Germination was slightly stimulated at 50 and 100 ppm, while at 250,500 and 1000 ppm, the final germination percentage was significantly reduced by simazine. Atrazine also retarded the rate of germination and suppressed the final percentage at 250 to 1000 ppm. Like the triazines, 2, 4-D also delayed germination, but it showed greater phytotoxicity than the triazines. At 5 ppm 2, 4-D promoted the rate as well as the final percentage of germination. Nevertheless, at higher concentrations (100 to 1000 ppm) 2,4-D remarkably decreased the rate and final percentage of germination. Furthermore, the reduction in final germination percentage was considerably greater in the dark than in light at comparable dosages.

EFFECT OF HERBICIDES ON SHOOT GROWTH

(a) Shoot growth in light: Results of seedling development are given in Table 2. Simazine significantly inhibited shoot growth at all the concentrations used. The action of atrazine in comparision to simazine was more adverse on shoot development, particularly at higher concentrations where it reduced the shoot length to about one-third of control. Essentially similar results were obtained with 2, 4-D, which proved to be more inhibitory than either of the triazines (Table 2).

(b) Shoot growth in dark: Simazine did not inhibit shoot growth significantly in dark except at 1000 ppm. However, atrazine considerably retarded shoot growth at higher concentrations but slightly stimulated at 5 ppm. Shoot elongation was stimulated

by 2, 4-D at 5 and 50 ppm but was retarded at concentrations greater than 250 ppm (Table 2).

EFFECT OF HERBICIDES ON ROOT GROWTH

(a) Root growth in light: Under normal conditions, in soil, three seminal roots are produced when a rice seed germinates. However, when seeds are germinated in Petri dishes on filter paper, usually more than three seminal roots are produced (Juliano & Aldama, 1937); out of these roots, one (primary root) develops directly from the radicle followed by two others.

Simazine retarded the root growth in light. The number and average length of seminal roots formed in higher concentrations (250 to 1000 ppm) was lesser than the control. Higher concentrations of simazine also decreased the growth of primary root. Like simazine, atrazine adversely affected the elongation of primary root and the number and average length of seminal roots were decreased. Unlike the triazines, 2-4-D completely arrested the root growth at all concentrations (Table 2).

(b) Root growth in dark: Both the triazines affected root growth more or less as adversely as they did in light (Table 2). The length of primary root and number and average length of seminal roots were all reduced in seeds treated with simazine and atrazine. Inhibitory effect increased with the increase in concentration. The behaviour of 2, 4-D was identical as in light, it completely checked the formation of roots.

Discussion

Although simazine and atrazine did not affect seed germination in light, both of them showed an inhibitory effect in dark at higher concentrations. In the earlier studies, the effect of triazines on seed germination was usually investigated in light (Gast *et al*, 1956, Grover, 1962; Sasaki & Kozlowski, 1968a). However, Saski & Kozlowski (1968b) studied the effect of three triazines on germinations of *Pinus resinosa* Ait., in light as well as in dark, but only 5 and 50 ppm concentrations were used and they were unable to find any inhibitory effect on seed germination.

The depressive effect of moderate and high dosages of 2-4,-D and stimulatory effect at low concentration, observed in the present study, correspond with the earlier results (Rojas-Garciduenas *et al*, 1962, Kozlowski & Sasaki, 1968). Greater inhibition of germination by 2,4-D in dark as compared with light, at higher doses, supports the hypothesis of Evenari (1957) who demonstrated that light partially abolishes the inhibiting action of 2-4-D.

The reduction in shoot growth in light by the triazines can be explained on the grounds that they inhibit non-cylic photophosphorylation and consequently photo synthesis (Moreland *et al*, 1959; Bishop, 1962). Further, it is a well known fact that the factors which influence photosynthesis also consequently affect plant growth (Sweet & Wareing, 1966. The inhibition of photosynthesis in light led to reduction in shoot growth due to scarcity of assimilates. Although relatively lesser degree of reduction of shoot growth by triazines in dark was obseryed yet it indicates that certain physiological processes other than photosynthesis also affected by traiazines Jordan *et al*, 1966).

TABLE 1. Percentage germination of seeds of *Oryza sativa* L. after treatment with herbicides (standard errors are given against the values).

Treatments	Concentration (ppm)	DAYS AFTER TREATMENT									
		3		5		9		12		15	
		L	D	L	D	L	D	L	D	L	D
Control	—	1 ±1.6	0	42 ±6.4	2.5±1.4	75 ±1.6	15 ±7.0	82 ±3.2	25 ±3.5	82±3.2	34 ±2.2
Simazine	5	5 ±2.9	0	30.5±4.5	2.5±1.4	34 ±7.3	22.5±3.2	37 ±3.2	30 ±4.5	90 ±3.5	30 ±4.5
	50	2.5±2.5	0	37.5±3.2	0	82.5±4.3	20 ±2.0	85 ±5.0	31 ±5.0	87.5±4.3	34 ±3.2
	100	7.5—3.2	0	42.5±6.0	2.5±1.4	77.5±3.2	14 ±3.2	82.5±3.2	31 ±2.3	82.5±3.2	37.5±4.7
	250	0	0	45 ±6.4	0	67.5±6.0	22.5±5.1	80 ±3.5	25 ±3.5	80 ±3.5	25 ±3.5
	500	0	0	42.5±6.4	1.0±1.0	82.5±5.1	22.5±3.2	82.5±5.1	25±3.5	82.5±5.1	25±3.5
	1000	0±0	0	32.5±4.7	0	70 ±4.5	14 ±2.3	77.5±6.0	22.5±3.2	77.5±6.0	22.5±3.2
Atrazine	5	1 ±1.0	0	42.5±5.0	25 ±1.4	77.5±6.6	14 ±2.3	82.5±6.0	22.5±5.2	82.5±2.0	31 ±3.2
	50	0	0	30.5±3.5	5 ±2.0	70 ±7.3	17.5±3.2	82.5±3.2	31 ±3.2	85 ±2.0	34 ±5.5
	100	0	0	12.5±3.2	0	75 ±4.5	14 ±1.2	82.5±4.2	25 ±2.0	82.5±4.2	27.5±4.3
	250	0	0	30 ±4.5	0	52.5±4.7	12.5±4.3	75 ±6.1	22.5±4.3	77.5±6.0	22.5±4.3
	500	1 ±1.0	0	42.5±5.1	2.5±1.4	67.5±7.2	14 ±4.3	75 ±6.1	22.5±3.2	77.5±6.0	25 ±3.5
	1000	0	0	17.5±3.2	0	63 ±5.8	12.5±3.4	75 ±6.3	17.5—4.2	82.5±7.3	22.5±3.5

2,4-D	5	12.5±4.3	0	50 ±7.3	2.5±1.4	75 ±7.3	12.5±3.2	87 ±4.3	25 ±4.1	92.5±1.4	52.4±3.2
	50	20 ±4.5	0	40 ±4.5	2.5±1.4	90 ±2.0	7.5±1.4	92.5±1.4	25 ±2.8	92.5±1.4	32.5±5.2
	100	0	0	20 ±2.0	0	57.5±6.6	5 ±2.0	60 ±2.0	12.5±3.2	60 ±2.0	12.5±3.2
	250	0	0	22.5±3.2	5 ±2.9	47.5±3.2	7.5±2.5	47.5±3.2	10 ±1.4	47.5±3.2	10 ±1.4
	500	0	0	2.5±1.4	2.5±1.4	5 ±2.0	2.5±1.4	5 ±2.0	2.5±1.4	±5 2.0	2.5±1.4
	1000	0	0	0	0	0	0	0	0	0	0

$LSD_{0.05}$ = 3.51, $LSD_{0.01}$ = 4.61, $LSD_{0.001}$ = 5.89

Analysis of Vajiance of Gejmination Data

Source of Variation	SS	df	MS	F
Treatments	3758	293	12.83	2.66PPP
Residual	2841	588	4.83	—
Total:	6599	881	—	—

TABLE 2. Effects of herbicides on root and shoot growth of *Oryza sativa* L. (16 days after treatment). Standard errors are given below the values.

Treatment		'No. of roots' (excluding main root)		Average length of all roots		Length of main root (largest root)		Length of shoot	
		L	D	L	D	L	D	L	D
Control		+ 2.83 — 0.066	+ 1.42 — 0.048	+ 4.92 — 0.32	+ 3.07 — 0.27	+ 4.44 — 0.26	+ 5.25 — 0.30	+ 3.58 — 0.33	+ 1.83 — 0.18
Simazine	5ppm	+ 2.70* — 0.034	+ 1.50n.s. — 0.042	+ 4.20 — 0.26	+ 2.72 — 0.31	+ 4.35n.s. — 0.18	+ 3.12** — 0.32	+ 2.30** — 0.26	+ 1.60n.s. — 0.27
,,	50 ,,	+ 2.73* — 0.053	+ 1.27** — 0.059	+ 4.25* — 0.37	+ 1.78*** — 0.30	+ 5.16n.s. — 0.33	+ 2.00*** — 0.27	+ 2.53** — 0.31	+ 1.57n.s. — 0.24
,,	100 ,,	+ 2.86 — 0.056	+ 1.28** — 0.042	+ 3.68** — 0.45	+ 2.23** — 0.25	+ 3.76** — 0.28	+ 2.80*** — 0.31	+ 2.84** — 0.27	+ 1.60n.s. — 0.20
,,	250 ,,	+ 2.56** — 0.62	+ 1.23** — 0.037	+ 3.84** — 0.21	+ 2.11** — 0.42	+ 3.94* — 0.30	+ 2.56*** — 0.25	+ 2.56** — 0.21	+ 1.53n.s. — 0.23
,,	500 ,,	+ 2.42*** — 0.039	+ 1.14*** — 0.039	+ 3.96** — 0.19	+ 2.02*** — 0.29	+ 4.01* — 0.16	+ 2.33*** — 0.20	+ 2.41** — 0.18	+ 1.44n.s. — 0.27
,,	1000 ,,	+ 2.25*** — 0.054	+ 1.18*** — 0.051	+ 2.68*** — 0.35	+ 1.82*** — 0.17	+ 3.12** — 0.27	+ 2.16*** — 0.23	+ 2.26*** — 0.24	+ 1.38* — 0.15
Atrazine	5 ,,	+ 2.50** — 0.043	+ 1.37n.s. — 0.040	+ 3.69** — 0.22	+ 2.54* — 0.24	+ 4.31* — 0.32	+ 3.00*** — 0.33	+ 2.80** — 0.27	+ 2.36* — 0.18
,,	50 ,,	+ 1.2*0** — 0.030	+ 1.24*** — 0.035	+ 1.26*** — 0.33	+ 2.43* — 0.20	+ 1.50*** — 0.25	+ 2.83*** — 0.28	+ 1.39* — 0.22	+ 1.41n.s. — 0.22
,,	100 ,,	+ 1.15*** — 0.043	+ 1.25** — 0.044	+ 1.30*** — 0.24	+ 1.88*** — 0.26	+ 1.62*** — 0.21	+ 1.97*** — 0.18	+ 1.12*** — 0.23	+ 0.80*** — 0.16
,,	250 ,,	+ 1.23*** — 0.029	+ 1.18* — 0.038	+ 1.31*** — 0.18	+ 1.55*** — 0.24	+ 1.54*** — 0.18	+ 1.8?*** — 0.21	+ 1.23*** — 0.25	+ 0.74*** — 0.13
,,	500 ,,	+ 1.17*** — 0.036	+ 1.20*** — 0.026	+ 1.23*** — 0.21	+ 1.19** — 0.17	+ 1.39*** — 0.20	+ 1.30*** — 0.23	+ 1.19*** — 0.21	+ 0.65*** — 0.19
,,	1000 ,,	+ 1.01*** — 0.041	+ 1.08*** — 0.033	+ 1.17*** — 0.32	+ 0.98*** — 0.21	+ 1.31*** — 0.23	+ 1.27*** — 0.20	+ 1.17*** — 0.18	+ 0.67*** — 0.15

2,4—D	5 ,,	+	0***	+	0***	+	0***	+	0***	+	0***	+	0***	+	0.86***	+	2.44*dd*
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.28
,,	50 ,,		0***		0***		0***		0***		0***		0**		0.84***	+	2.43**
			—		—		—		—		—		—		—	—	0.24
,,	100 ,,		0***		0***		0***		0***		0***		0***		1.01***	+	1.62us..
			—		—		—		—		—		—		—	—	0.19
,,	250 ,,		0***		0***		0***		0***		0***		0***		0.51***	+	1.58*
			—		—		—		—		—		—		—	—	0.10
,,	500 ,,		0***		0***		0***		0***		0***		0***		0.56***	+	1.00***
			—		—		—		—		—		—		—	—	0.14
	1000 ,,		0***		0***		0***		0***		0***		0***		0***		0***
			—		—		—		—		—		—		—		—

L — Light
D — Dark

Level of Significance
* p <.05
** p <.01
*** p <.001 n.s. non-significant

Like the triazines, 2-4-D also adversely affected the shoot growth in light at 50 to 1000 ppm. The stimulation of shoot growth caused at 5 ppm by 2-4-D in dark could be explained by the findings of Huffaker *et al*, (1962) who demonstrated that the dark fixation of CO_2 catalyzed by the phosphoenol pyruvate reaction systems were increased at low doses of 2,4-D which stimulated shoot growth. Furthermore, low concentrations of 2-4-D induce an increase in the respiration rate (Humphrey & Dugger, 1957) and this factor can also account for the increase in shoot growth at 5 ppm the promotion of respiratory rate can be due to accumulation of coumarin in the tissues induced by 2, 4-D (Van Overbeck *et al*, 1951) since some concentrations of this substance cause such an effect.

Both the triazines inhibited root growth in light as well as in dark. Presumably this was due to the phytohormonal activity of the triazines, since it is well known that auxins at higher concentration inhibit root growth. Similarly the suppression of root growth observed could be due this type of phytohormonal action of triazines. Moreover, the inhibition of root growth (particularly in dark) indicates, as pointed out earlier, that other processes entirely independent of the photosynthetic inhibition are also involved in the herbicidal action of triazines.

The complete inhibition of root development by 2-4-D is supposed to be due to its strong phytohormonal action (Audus, 1959). Although, decrease in root growth by 2, 4-D has been found by several workers Eliasson & Palen, 1972), yet complete blockage of root growth seems to be previously unreported. The results of the present investigation did not give a definite indication of the phytohormonal action of triazines and further investigations are needed in this direction which may resolve the controversy.

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References

Audus, L.J. 1959. Plant growth substances. 2nd ed. Leonard Hill Books Ltd., London.

Bishop, N.J. 1962. Inhibition of the oxygen evolving system of photosynthesis by amino-triazines. Biochem. biophys. Acta, 71 186-189.

Copping, L.G, D.E. Davis, and C.G. Pillai. 1972. Growth regulator-like activity of atrazine and ametryne. Weed. Sci., 20: 274-277.

Eliasson, L and K. Palen. 1972. Effect of light on the response of pea seedling roots to 2,4-D. Physiol. Plant., 28: 206 209.

Evenari, M. 1957. The physiological action and biological importance of germination inhibitors. In: Biological action of growth substances. Symp. Soc. Expt. Biol., 11: 21-43. Cambridge University Press.

Gast, A., E. Knusli, and H. Gysin. 1956. Uber pflanzenwachstumsregulatoren: uber weitre phytotoxische Triazene. Experientia, 12: 146-148.

Grover, R. 1962. Germination of three coniferous species after treatment with simazine. Weeds, 10: 246.

Huesuh, Y.L. and C.H. Lou. 1947. Effects of 2, 4-D on seed germination and respiration. Science, 105: 283-285.

Huffaker, R.C. M.D. Millier, and D.S. Mikkelson. 1962. Effect of 2,4-D iron and chelate supplements on dark CO2 fixation in cell-free homogenates of field beans. Crop. Sci., 2: 127-129.

Humphrey, T.E. and W.M. Dugger, Jr. 1957. The effect of 2, 4-D on the respiration of etiolated pea seedlings. Pl. physiol., 32: 530-536.

Jordan, D.S, T. Murashige, J.D. Mann, and B.E. Day. 1966. Effect of photosynthesis inhibiting herbicides on non-photosynthetic tobacco calus tissue. Weeds, 11I 134-136.

Juliano, J.B. and M.J. Aldama. 1937. Morphology of *Oryza sativa* L. Phillip. Agricult., 26: 1-134.

Kozlowski, T.T. and S. Sasaki. 1968. Germination and morphology of red pine seeds and seedlings in contact with EPTC, CDEC, CDAA, 2, 4-D and Picloram. Am. Soc. Hort. Sci., 193: 655-662.

Moreland, D.E., W.A. Genter, J.L. Hilton, and K.L. Hill. 1959. Studies on the mechanism of herbicidal action of 2-chloro-4, 6-bis (ethylamino) S-triazine. Pl. Physiol., 34: 432-435.

Overbeek, J. Van. J.R. Blondeau, and V. Horne, 1951. Difference in activity between 2, 4-D and other auxins and the significance in herbicidal action. Pl. Physiol., 26: 687-695.

Robert, H.E. 1961. Dormancy in rice seeds. II. The influence of covering structures. J. Exp. Bot., 12: 430-445.

Rojas-Garciduenas, M., M.A. Ruiz, and J. Carillo. 1962. Effect of 2, 4-D and MCPA on germination and early growth. Weeds., 10: 69-71.

Sasaki, S. and T.T. Kozlowski. 1968a. Effect of herbicides on seed germination and early seedling development of *Pinus resinosa*. Bot Gaz., 129: 238-246.

Sasaki, S. and T.T. Kozlowski. 1968b. The role of cotyledons in early development of pine seedlings. Can. J. Bot., 46: 1173-1183.

Sasaki, S., T.T. Kozlowski and J.H. Torrie. 1968. Effect of pretreatment of pine seeds with herbicides on seed germination and growth of young seedlings. Can. J. Bot., 46: 255-262.

Sweet, G.B. and P.F. Wareing. 1966. Role of plant growth in regulating photosynthesis. Nature, 210: 77-79.

Taylor, L. 1942. Influence of oxygen tension on respiration fermentation and growth in wheat and rice. Am. J. Bot., 29: 721-738.

Wakonig, R. and J.T. Arnason. 1958. Effect of triazines on Chromosomes. Proc. 10th Intern. Congr. Genetics, Montreal., 2: 205.