TECHNICAL PAPERS

Effects of 2,4-D on Seed Germination and Respiration

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Synthetic plant-growth-promoting substances, known also as auxins, have been found to possess many practical applications in agriculture. They have been extensively used to prevent premature shedding of fruits, to induce rooting of cuttings, to induce parthenocarpy and ensure fruiting, to keep the bud in dormancy, and to improve the well-being of a plant in general (*4*). The reasons for the versatility of auxin treatment are still being conjectured. Only at very high dilutions (1 ppm) does indole acetic acid promote growth and accelerate protoplasmic streaming in the *Avena* coleoptile; at slightly higher concentrations (10–100 ppm) it depresses growth, stunts the plant, but induces root formation; and at still higher (100–1,000 ppm) it becomes destructive to chlorophyll and to the plant as a whole (*1*). The recent successful introduction of di- or trichlorophenoxyacetic acid, a compound which possesses high auxin activities, as a translocated herbicidal spray by F. D. Jones and others has amply revealed the importance of this new phase of research on auxin treatment. Furthermore, it has been shown that the herbicidal effect of this and allied chemicals on plants can be differential and selective, *i.e.* it can be applied effectively in the field to kill some major weeds while leaving the crops unharmed (*2*).

The facts that 2,4-D can be actively absorbed by, and freely translocated in, the plant before the latter is killed, and that its lethal effect on plants can be differential, seem to us most interesting and deserving of some physiological investigation.

Germination of seeds involves many of the physiological and biochemical processes exhibited in mature plants, such as the breakdown and translocation of the stored food, the formation of seedling organs, and the high respiratory and metabolic activities during germination. If a chemical compound happens to be injurious to certain plants and if it happens to be involved in inhibiting some specific metabolic activity therein, its physiological effect can be conveniently put to test by treating germinating seeds with such a chemical, the dosage of which may be easily controlled. Various kinds of seeds, differing in structure, in their principal food constituents, and in their biochemical behavior, can be employed for the test. These differences may be expected to manifest themselves through differences in their reaction toward a given chemical treatment. This procedure was employed by us to study the physiological action of 2,4-D on plants. The chemical treatment of seeds usually results in a complete inhibition of, or a substantial delay in, germination, which, on refined analysis, may reveal some specific metabolic disturbance in the seeds.

Seeds of barley and rice were first chosen as test materials. These cereal grains, which are more or less alike in structure and food content, differ physiologically in that rice excels in fermentative faculty and is alone able to germinate in the absence of oxygen (*3*). Seeds of uniform size and appearance were selected from the pure line stock kindly furnished us by the Department of Plant Pathology, Tsing Hua University, and then divided into lots of 100 seeds each. The different lots of seeds were treated with various dilutions of 2,4-D ranging from 0.0035 to 0.1 per cent. After 24 hours of treatment, each lot of seeds was thoroughly washed with water to remove the adhering chemical and put in a labeled Petri dish in which a moist filter paper had been placed. The Petri dishes were assembled in a moist chamber maintained at 25 ± 2°C. and the rate of germination of the seeds followed at suitable intervals. As long as the filter paper in the dish is kept moist, addition of excess water is avoided. In these experiments

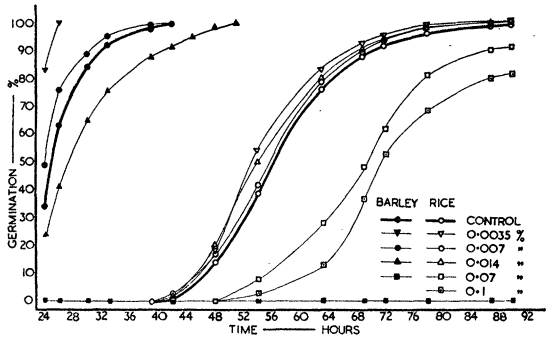

FIG. 1. The germination of barley and rice as affected by treatment with different concentrations of 2,4-D.

the criterion for germination used by Taylor (*3*) was adopted, *i.e.* seeds were regarded as germinated when any organ had attained a length of not less than 1.5 mm. The subsequent growth of the seedling was not included in our observations. The 2,4-D employed in these experiments was synthesized in our laboratory.

The rates of germination of the treated seeds are plotted in Fig. 1. Similar to other auxins, 2,4-D accelerates seed germination at low concentrations but delays it when a certain threshold of concentration is exceeded. The outstanding feature of our results is that the threshold concentration in retarding germination is higher in rice (ca. 0.07 per cent) than in barley (ca. 0.01 per cent). Furthermore, 0.07 per cent 2,4-D inhibits barley germination completely while it merely delays germination in rice. Even when the concentration is raised to 0.1 per cent, 2,4-D fails to prevent the rice from germinating, though it delays the process still further. Comparison of the germination rate of seeds treated with 2,4-D with that of seeds kept under aerobic and anaerobic conditions reveals a striking resemblance between the treated seeds and those under anaerobic condition. It seems as if oxygen were no longer available to

the treated seeds, so that the germination of barley, a typical aerobic seed, is entirely inhibited and that of rice, a seed known to be able to germinate anaerobically, is only duly delayed. Fig. 2 illustrates this point very well. The anaerobic

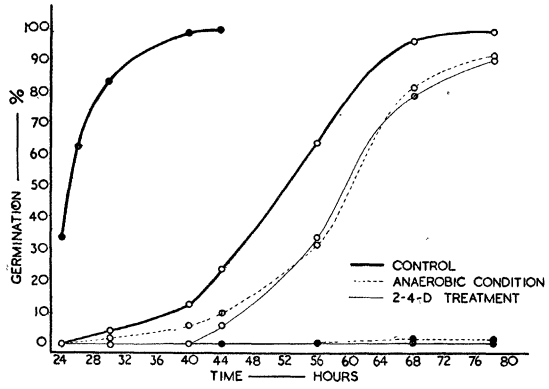

FIG. 2. Influence of 2,4-D treatment (0.1 per cent) and anaerobiosis on germination of barley and rice (barley = ●; rice = ○).

condition was attained by flushing the container of the germinating seeds with nitrogen.

The same procedure was extended to various other types of seeds—those which store their food in cotyledons (legumes) and those rich either in proteins (soybean) or in fats (*Raphanus*). Table 1 summarizes our findings. With the exception of sesame seeds, which may have a lower threshold of inhibition than the concentration employed, the results seem to substantiate our view regarding 2,4-D treatment.

For a final proof, gas exchange in the treated seeds of barley and rice was analyzed with Warburg microrespirometers. The aerobic (respiration) and anaerobic (fermentation) activities of the treated seeds during germination were calculated from the data on the assumption that the aerobic respiratory activity of the treated seeds was the same as that of the control, *i.e.* in both barley and rice, the R. Q. value is close to unity. The respiratory intensity was represented by CO_2 evolved in aerobic respiration, the value of which was obtained in the case of treated seeds by subtracting fermentation CO_2 from the total CO_2 evolved. The results are presented in Table 2. All values given are relative.

TABLE 1

	Percentage germination under anaerobic condition	Percentage germination of seeds treated with 0.1% 2,4-D
Rice	100	100*
Barley	0	0
Wheat	0	0
Soybean	0	0
Broad bean	0	0
Peanut	0	0
Raphanus	8–10	20–30*
Sesame	15–20	0

* No subsequent growth.

TABLE 2

RESPIRATORY ACTIVITIES OF BARLEY* AND RICE† TREATED WITH 0.1 PER CENT 2,4-D DURING EARLY PERIOD OF GERMINATION

Time (days)	Seeds	Experimentally determined: O_2 uptake, Total	O_2 uptake, Treated/Untreated	Experimentally determined: CO_2 evolution, Total	CO_2 evolution, Treated/Untreated	Calculated from values on experimentally determined activities: Respiration CO_2 evol. (%)	Fermentation CO_2 evol. (%)	Fermentation CO_2 evol. / Respiration CO_2 evol.	CO_2:O_2	Respiratory intensity Treated/Untreated
				Barley						
1st day	Untreated	12.0	0.33	11.0	1.36	100	—	—	0.92	0.34
	2,4-D-treated	4.0		15.0		33	103	3.12	3.75	
2nd day	Untreated	17.0	0.30	16.0	0.94	100	—	—	0.94	0.29
	2,4-D-treated	5.0		15.0		29	65	2.24	3.0	
3rd day	Untreated	57.0	0.21	52.0	0.30	100	—	—	0.91	0.20
	2,4-D-treated	12.0		15.0		21	9	0.42	1.25	
				Rice						
3rd day	Untreated	8.0	0.31	7.5	1.13	100	—	—	0.94	0.28
	2,4-D-treated	2.5		8.5		31	82	2.65	3.40	
6th day	Untreated	19.5	0.51	18.5	0.81	100	—	—	0.94	0.53
	2,4-D-treated	10.0		15.0		48	33	0.70	1.50	

*† Divisions on Warburg's microrespirometer scale/90 minutes/6 seeds and /60 minutes/10 seeds, respectively.

The low ratios in respiratory intensity between the treated and untreated seeds clearly indicate that the seeds treated with 2,4-D cannot very well utilize oxygen in the air during germination and must find some other recourse, such as fer-

mentation, for energy supply. Hence, during the few days following the chemical treatment, these seeds, in comparison with the control, exhibit a low oxygen uptake and a high carbon dioxide evolution, and, consequently, a high CO_2:O_2 ratio. This effect is very similar to that caused by germinating seeds under low oxygen tension, which has been fully described by Taylor (*3*). As a result of O_2 deficiency in the medium, aerobic respiration is reduced, and this reduction is to some extent compensated for by the fermentative activity of the germinating seeds, as evidenced by the high CO_2:O_2 ratios under such a condition. If they cannot furnish the energy necessary for germination fermentatively, most seeds will fail to germinate at all. If, as in rice, the seed is especially gifted with a highly functional fermentative mechanism, it can proceed to germinate, although with some delay, even after 2,4-D treatment or under anaerobic condition. However, this condition cannot go on indefinitely. Even rice cannot continue to grow in the complete absence of oxygen.

During recent years evidence has accumulated which shows that auxin is involved in the 4-carbon acid respiratory system (*4*). The increased respiration due to auxin treatment was found to parallel the increase in elongation of, and the acceleration of protoplasmic streaming in, the *Avena* coleoptile. This statement is probably true only at certain low concentrations of auxin.

Our experiments have shown that 2,4-D, at low concentrations (0.01 per cent), promotes germination; but, at higher concentrations (0.1 per cent), it begins to inhibit aerobic respiration and checks germination.

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Prevention of Respiratory Embarrassment in Therapeutic Curarized Convulsions

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Transitory asphyxia and cyanosis occur so frequently in spontaneous and therapeutically induced convulsions that cerebral anoxia was cons dered a possible explanation for the beneficial effects of convulsions in mental disorders. However, it has been demonstrated that the production of cerebral anoxia by the inhalation of nitrogen has no such therapeutic value (*4*). On the contrary, asphyxia contributes clinically to cardiac strain and plays a role in the fatalities and near-fatalities that occasionally occur in convulsions. The treatment of asphyxia by the use of chemical stimulants has proved futile (*3*). Administration of oxygen is ineffectual unless the air passages are open. The presence of trismus makes the installation of apparatus to clear the respiratory passages *after* a convulsion difficult (*1*) and often impossible. We have found that although administration of the convulsant electric current during inspiration facilitates postconvulsive breathing, since the first respiratory movement is then an expiration which clears the passages, this so increases intrapulmonary and venous pressure as to make the procedure not without risk. This procedure has, therefore, been discarded.

Certain few patients are "hard breathers" in that they persistently have asphyxial episodes during and after convulsive seizures. They seem to fall into two main categories: (1) those whose illness is characterized by agitation, depression, rejection of food and sleep, and other psychological "oral" qualities; and (2) patients with evidence of generalized arteriosclerosis or other neurologic complications, in whom there appears to be some inefficiency in the respiratory apparatus. The physician is sometimes compelled to treat a patient with coronary disease, whose agitation is so marked and dangerous to the heart as to necessitate the administration of shock treatment in an attempt to terminate the mental disorder. It is precisely those patients falling into both categories who are apt to show respiratory difficulties and in whom asphyxia is most dangerous. In these patients it is impossible to soften the convulsion adequately by means of curare because it increases respiratory difficulty and asphyxia. By premedication with sodium pentothal or sodium amytal (*2*) one can increase slightly the dosage of curare. The sedation seems to diminish the preconvulsive anxiety and restlessness and to diminish pharyngeal spasm. Unfortunately, these barbiturates increase the postconvulsive apnea.

We have found a simple means of avoiding asphyxia and consequently diminishing cardiac strain. With this technique a Guedel rubber airway is installed *during* the convulsion.

The patient is prepared as usual for the treatment. He is placed supine on a flat bed without hyperextension, since the latter increases respiratory difficulty. The barbiturate and curare, or curare alone, are injected. In electroshock convulsions, a cloth gag is placed between the teeth to protect the tongue and lips. The current is then applied. During the initial tonic flexion phase, the gag is forcibly bitten. This is followed by a moment of relaxation in which the mouth is opened widely. At this point a Guedel rubber airway is introduced to the hilt. Where metrazol is used, the cloth gag is not necessary. The initial movement of the mouth is an opening one, and the airway is similarly introduced. With the airway in place there may be respiratory exchange in the m dst of the convulsion. Before the end of the convulsion, the patient is turned on the side with the mouth down. Mucus and saliva will flow from the airway. Respiration usually begins shortly after the convulsion is over. The airway is removed a few minutes after the convulsion, when respirations are normal and the mouth and jaw relaxed.

With this technique asphyxia and cyanosis are now rare. There are occasional periods of apnea, which seem to be of central origin. In these apneic periods it is a simple matter, with the air passages open, to institute respiration, if one so desires, by abdominal pressure. The airway acts as an efficient mouth gag and bitten tongues and lips are infrequent. A theoretical objection to the method is the possibility of dislocation of the jaw and trauma to the pharynx, but in practice neither occurs. Postconvulsive headache, nausea, and confusion have been diminished considerably. Larger amounts of curare can be administered safely to hard breathers. In one patient with known severe coronary disease, the postconvulsive electrocardiographic changes were less marked and of much