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INFLUENCE OF LIGHT ON CHEMICAL INHIBITION OF LETTUCE SEED GERMINATION

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INTRODUCTION

It is well known that some lots of lettuce seed (*Lactuca sativa* L.), under particular environmental conditions, will give a higher percentage germination if exposed to light than if maintained in darkness during the germination test (Larson and Ure, 1924; Shuck, 1933, 1934; Flint, 1934*a*, 1934*b*, 1935, 1936; Flint and McAlister, 1937; Thompson, 1938). This response, which is found also in many other species and has been termed "light-sensitivity," is influenced by several factors including the variety, storage history of the particular seed lot, temperature and water supply during the test, and characteristics of the irradiation treatment. No systematic study of the role of the influential factors has been reported for lettuce seed, and there is very little information as to the mechanism of light-sensitivity in general. One suggested mechanism is that dormancy is caused by the presence in the seed of a growth inhibitor which is somehow rendered inactive by light.

There exists a considerable body of evidence that dormancy of a number of species is controlled, or at least influenced, by chemical inhibitors present in, or formed by, the seed or fruit (for literature citations see Stout and Tolman, 1941; Nutile, 1944, 1945). The presence of such inhibitors in lettuce has been invoked as an explanation for various aspects of dormancy (Borthwick and Robbins, 1928; Shuck, 1934), and evidence for the formation of germination inhibitors by this species has been reported by Shuck (1935) and by Stout and Tolman. Similar evidence, to be published separately, has been obtained by the writer.

Nutile (1944, 1945) discovered that in darkness low concentrations of coumarin prevented germination of lettuce seed under conditions

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favorable to germination in the absence of this compound. Appreciably higher concentrations were required for comparable inhibition if the seeds were illuminated, however.[2] Nutile suggested that coumarin, or related compounds, might occur naturally in lettuce seed and play the role of a germination inhibitor. Coumarin, which itself does not absorb visible radiation, was believed to become photosensitive upon entering the seed.

Two facts have been regarded as furnishing some support for the suggestion of coumarin as an endogenous inhibitor. In the first place the compound is known to occur in a considerable number of plant species (see Nutile, 1945), although its presence in lettuce has not been demonstrated. Secondly, a number of naturally occurring substances having inhibitive effects on cell growth in both plant and animal tissues have been shown to possess an unsaturated lactone structure as does coumarin. Coumarin has been found to inhibit germination and seedling development in several other species also (see Audus and Quastel, 1947), although the effect of light has not been investigated in these.

Nutile's hypothesis thus seems rather attractive, and if substantiated might contribute greatly to solution of the problem of light-sensitivity of seeds.

The present report describes some experiments undertaken with the purpose of ascertaining whether the effect produced by coumarin is specific for this substance or can be duplicated by other compounds more or less similar in molecular structure.

EXPERIMENTATION AND RESULTS

Methods.—All the data herein reported relate to the black-seeded variety Grand Rapids; the seeds were of the 1944 crop and had been in storage approximately 2 years at the time the experiments were

[2] As stated in Nutile's publications, the differential inhibition in light and dark was found when the substrate was moistened with 10 to 100 p.p.m. of coumarin. This is in agreement with our results. In an erratum supplied by Mr. Nutile it is stated, however, that the actual concentrations employed were only one-tenth as great as originally reported. This correction appears to have been made because the solubility of coumarin is sometimes given in the literature (Seidell, 1941; Hodgman, 1944) as 0.01 percent (100 p.p.m.), a value taken from a determination by Dehn (1917); examination of Dehn's paper reveals that the determination was of a very low order of precision. The solubility of coumarin was earlier reported (Schimmel & Co., 1899) as 0.12 percent at 0° C., 0.18 percent at 16-17°, and 0.27 percent at 29-30°; we have found the solubility at 25° to lie between 0.2 and 0.3 percent.

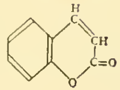
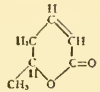
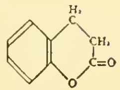
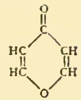
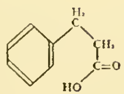
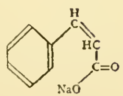
commenced. Seeds of this age were chosen so that there would be no significant change in germinative energy during the period of some months required for the study. Some of the tests were repeated with variety Black-Seeded Simpson, with similar results.

Two series of experiments were conducted. The first was designed to determine approximately the effective concentrations of the various substances and to evaluate the possible influence of variations in temperature, spectral quality and intensity of the light, duration of the germination period, and other details of technique. The general character of the results was found to be the same at temperatures from 16 to 23°, in red light and in white light, and over a hundredfold range of light intensity.

In the second group of tests a finer series of concentrations was compared, all other conditions being kept uniform. Each treatment was carried out in duplicate (100 seeds per dish) and the experiments were repeated one or more times at the critical concentrations. The air-dry seeds were distributed on dry blotters in small Petri dishes, amounts of the solutions slightly greater than required to saturate the blotters were added, and the dishes placed, within a few minutes, under the desired condition of light or darkness which was maintained until observations were made 3 or 4 days later. The water-jacketed thermostat employed as a germinator was provided with several independent compartments so that a number of tests could be made concurrently in light and in darkness without interference. The temperature was maintained at $22.55 \pm 0.05°$ C. Illumination was furnished by a 20-watt red fluorescent lamp 20 cm. above the seeds with a sheet of window glass interposed.

Results.—A number of compounds were found to inhibit germination to a greater extent in darkness than in light; with increasing concentration the germination was progressively less both in light and in dark, the inhibitory effect of a given concentration being always greater in darkness. Data obtained in the present study suggest that the concentration-inhibition curves for various compounds may differ greatly in shape. To assess the relative effectiveness of different substances it would be desirable to determine concentration-inhibition curves for each, so that comparison could be made either among the concentrations required for a given degree of inhibition, or of the effects produced by a selected concentration. A complication arises in experiments of this kind in that it is necessary to distinguish between effects upon the *initiation* of germination and those on *subsequent development* of the seedling. Several substances were found to permit

TABLE I.—*Effect of several toxic compounds on germination of Grand Rapids lettuce in light and in dark*

Compound	Formula	Concentration		Germination (as % of water control [1])	
		Molar	p.p.m.	In light	In dark
Coumarin		0.0002	29	99	31
Parasorbic acid		.0002	30	98	82
Melilotin		.0005	56	99	78
γ-Pyrone		.001	96	100	84
Sorbic acid	$CH_3-CH=CH-CH=CH-COOH$	.001	112	98	87
Hydrocinnamic acid		.002	300	99	88
Sodium *tr*-cinnamate		.002	340	100	77

[1] At 22.5° the water controls give somewhat greater germination in light than in dark; the averages of 10 experiments (2,000 seeds each in light and in dark) were: light 89.6 percent, dark 84.8 percent. In order to correct for this effect of light itself, the results in the presence of added chemicals have been calculated as percentages of the corresponding light or dark controls.

emergence and some elongation of the root and shoot but then to exert secondary toxic effects upon one or the other organ. A similar observation has been made by Audus and Quastel (1947) in the case of coumarin. As the criterion of germination used in this study is elongation of the seedling axis, a secondary suppression of such elongation, if sufficiently great, renders impossible the accurate determination of the germination percentage.

For this reason it was thought preferable to compare the lowest concentrations (within a twofold range) that showed a significant difference between light and dark. At such concentrations there was no

Table 2.—*Effect of several less active compounds on germination of Grand Rapids lettuce in light and in dark*

Compound	Molar concentration	Germination (as % of water control) In light	In dark
Sucrose	0.1	94	12
Maltose	.1	87	48
Lactose	.1	100	29
Glucose	.1	98	57
Fructose	.1	87	21
Galactose	.1	97	39
Mannose	.1	97	32
Arabinose	.15	90	56
Xylose	.15	94	30
Sorbitol	.1	94	45
Mannitol	.1	92	31
Ascorbic acid	.1	96	50
α-Alanine	.1	99	29
β-Alanine	.1	98	32

uncertainty in classifying a seed as germinated or not: a portion of the population showed no macroscopic development at all, while the remainder had radicles at least 4 to 5 mm. long.

In table 1 are listed, in descending order of activity, some compounds which, at approximately the same order of magnitude of concentration, act similarly to coumarin. In table 2 are listed several substances which are much less toxic than the foregoing, but which, in sufficiently high concentration,[3] also cause greater suppression of germination in darkness than in light.

[3] Whether the high osmotic pressure of these solutions is entirely responsible for their inhibitory effect is not known. Stout and Tolman (1941) found that comparable inhibition of New York No. 12 variety of lettuce required concentrations of sodium chloride or sucrose equivalent to 5 or 6 atmospheres.

DISCUSSION

Consideration of the studies of lettuce germination reported in the literature together with results of as yet unpublished experiments of the writer leads to the following view:

Dormancy and germinability of lettuce seed appear to be controlled by a delicately balanced mechanism which is highly sensitive to a diversity of factors. The response of a given lot is influenced both by the integrants of the environment at the time of testing and by the internal condition of the seed, which is determined in part by its history from the time of harvest or even earlier. The precise effect of each of the several operative external and internal elements is dependent upon the concurrent status of all the others.

Light is not essential for germination of lettuce. Depending upon conditions it may or may not be favorable. Germination of all the viable individuals of a given population can occur in darkness provided that the constellation of influential internal and external factors is optimal. When this is not the case, as in freshly harvested seed or at elevated temperature, the suppression of germination which is found in darkness can be overcome to some degree by illumination.

Similarly, light tends to counteract the adverse influence of exogenous chemical germination inhibitors. The foregoing results demonstrate that this behavior is independent of the specific inhibitor. In other words, stimulation by light, which obviously can be manifested only under conditions which are suboptimal for germination, is a general response to such conditions.

The finding that chemical induction of light-sensitivity is not a specific effect of coumarin, or even of compounds possessing similar molecular groupings, would appear to weaken materially, although not necessarily to disprove, the hypothesis that coumarin plays a role in the natural dormancy of lettuce seed.

SUMMARY

It has been proposed that coumarin may be the endogenous germination inhibitor responsible for light-sensitivity of lettuce seed, on the basis of the finding that the inhibitory action of this substance is greater in darkness than in light. In the present paper it is shown that the effect is not specific for coumarin. Other compounds demonstrated to act similarly are parasorbic acid, melilotin, γ-pyrone, sorbic acid, hydrocinnamic acid, sodium *trans*-cinnamate, sucrose, maltose, lactose, glucose, fructose, galactose, mannose, arabinose, xylose, sorbitol, mannitol, ascorbic acid, α-alanine, and β-alanine.

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LITERATURE CITED

Audus, L. J., and Quastel, J. H.
1947. Coumarin as a selective phytocidal agent. Nature, vol. 159, pp. 320-324.

Borthwick, H. A., and Robbins, W. W.
1928. Lettuce seed and its germination. Hilgardia, vol. 3, pp. 275-305.

Dehn, W. M.
1917. Comparative solubilities in water, in pyridine and in aqueous pyridine. Journ. Amer. Chem. Soc., vol. 39, pp. 1399-1404.

Flint, L. H.
1934*a*. Light-sensitivity in relation to dormancy in lettuce seed. Proc. Intern. Seed Testing Assoc., vol. 6, pp. 487-489.
1934*b*. Light in relation to dormancy and germination in lettuce seed. Science, vol. 80, pp. 38-40.
1935. Sensitivity of dormant lettuce seed to light and temperature. Journ. Washington Acad. Sci., vol. 25, pp. 95-96.
1936. The action of radiation of specific wave-lengths in relation to the germination of light-sensitive lettuce seed. Proc. Intern. Seed Testing Assoc., vol. 8, pp. 1-4.

Flint, L. H., and McAlister, E. D.
1937. Wave lengths of radiation in the visible spectrum promoting the germination of light-sensitive lettuce seed. Smithsonian Misc. Coll., vol. 96, No. 2, 8 pp.

Hodgman, C. D., editor.
1944. Handbook of chemistry and physics. 28th ed. Cleveland.

Larson, A. H., and Ure, R.
1924. Some factors affecting the germination of lettuce seed. Minnesota Stud. in Plant Sci., vol. 1, pp. 289-294.

Nutile, G. E.
1944. Studies on the germination of lettuce seed. Inducing dormancy with coumarin. Proc. Assoc. Off. Seed Anal. N. Amer., 35th Ann. Meet., pp. 120-135.
1945. Inducing dormancy in lettuce seed with coumarin. Plant Physiol., vol. 20, pp. 433-442.

Schimmel & Co.
1899. Semi-annual report. April, p. 66.

Seidell, A.
1941. Solubilities of organic compounds, vol. 2. New York.

Shuck, A. L.
1933. Some suggestions for the prevention of erratic germination of lettuce seed. Proc. Assoc. Off. Seed Anal. N. Amer., 26th Ann. Meet., pp. 284-285.

1934. Some factors influencing the germination of lettuce seed in seed laboratory practice. New York State Agr. Exp. Stat., Techn. Bull. 222, 21 pp.

1935. The formation of a growth inhibiting substance in germinating lettuce seeds. Proc. Intern. Seed Testing Assoc., vol. 7, pp. 9-14.

Stout, M., and Tolman, B.

1941. Factors affecting the germination of sugarbeet and other seeds, with special reference to the toxic effects of ammonia. Journ. Agr. Res., vol. 63, pp. 687-713.

Thompson, R. C.

1938. Dormancy in lettuce seed and some factors influencing its germination. U. S. Dep. Agr. Techn. Bull. No. 655, 20 pp.