THE EFFECTS OF INDIVIDUAL ENVIRONMENTAL FACTORS ON THE CHEMICAL CONSTITUENTS OF PLANTS.

I. GLUCOSIDE OF FLAX.*[1]

BY NOEL M. FERGUSON.[2]

INTRODUCTION.

For many years the interest of a considerable number of plant physiologists has been centered on the rôle played by certain climatic factors upon the chemical constituents of plants.

Among the earlier workers might be mentioned Collins and Blair (1) whose experiments tended to prove that certainly temperature and perhaps moisture as well were effective in varying the chemical constitution of flax. They found that seeds of flax plants which were grown in hot, dry climates yielded a higher quantity of HCN than seeds obtained from plants growing in temperate regions. Then followed a number of investigations dealing with glucosidal and alkaloidal plants other than flax. Among the more recent were two researches which have a direct bearing on the effect of ultraviolet light upon the glucosides in *Digitalis purpurea L.*

The first of these is the work of Miss McCrea (2) which deals with the effects of ultraviolet light upon the glucosidal content of *Digitalis purpurea L.*, in which it is claimed that plants grown under Vitaglass for a given length of time and then placed in the field produced more physiologically active constituents than those grown under window glass and then placed in the field.

The second research on *Digitalis purpurea L.*, carried out by Arthur and Leonard (3) which tends to disprove that of Miss McCrea, does not appear to have been successful since the comparisons are unconvincing. Due to the fact that the two experiments were carried out under somewhat different conditions and in both investigations there were uncontrolled factors, a comparison is impossible.

With these facts in mind the writer became interested in knowing just what effect the climatic conditions, moisture and light, and artificial light of certain wavelengths have in changing the glucosidal content of flax.

EXPERIMENTAL.

The flax was sown in flats and allowed to germinate under ordinary greenhouse conditions. For each series of sets thirty flats were prepared of which the twenty-five healthiest were used. Five of these served for the low moisture experiment and will be designated hereafter as Set "A," five for high moisture, designated as Set "B," five for the control condition, designated as Set "C," five for the low light, designated as Set "D" and five flats for the high light, designated as Set "E."

In all there were four series of sets, the last of which was used for the raying experiment. These series of sets were prepared sixteen days apart so as to have them growing over a long period of time, thus ruling out any variations which might exist in temperature and length of day. The

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[2] Assistant Professor of Botany and Pharmacognosy, St. Louis College of Pharmacy.

plants were all grown under the same conditions of temperature and humidity. The average temperature was about 65° C.

Sets "A," "B" and "C" were placed in an east room in the greenhouse which was not an end room. Therefore, any light which came in from the north, south or west had to pass through two thicknesses of glass. The sets were rotated within this room so that all of them received an equal supply of light. Sets "D" and "E" were placed in the end room adjoining the room containing Sets "A," "B" and "C" and consequently received more light since one side faces east and the end south. Here also the flats in each set were rotated in order to insure the plants an equal supply of light.

Assays were run on each series of sets for three consecutive weeks, beginning two weeks after germination, *i. e.*, one week after the experiment was started.

The amount of moisture added to Set "A" and Set "B" was varied from that added to the control set by paying particular attention to the condition of the plants in these sets. Set "B" was much greener than Set "A," and showed less tendency to wilt. The percentage of moisture in the soil of these three sets was also determined and the amount of water was added in such quantities as to give Set "A" a very small amount of soil moisture, Set "B" a great amount of soil moisture and the control set, Set "C," an amount midway between that found in Sets "A" and "B."

That the method of watering produced different water contents of the plants is shown by the following table of dry-weight percentages of the three sets.

TABLE I.

Series.	Set "A."	Set "B."	Set "C."
I	21.0 %	13.47%	15.96%
II	13.4 %	9.7 %	11.8 %
III	13.96%	10.8 %	11.65%
III_2	13.07%	8.3 %	9.6 %

In Sets "D" and "E" the light conditions were varied, thereby also changing the rate of transpiration and consequently the percentage of soil moisture.

During the germination period and up to the time the experiment was started, all of the plants received the same amount of light and moisture. The temperature conditions were also the same. In order to reduce the variations to a minimum the plants were rotated about the room and particular attention was paid to the brightness of the day in order that each plant might receive the same amount of light.

At the beginning of the experiment the flats known as Set "D," were placed under cheesecloth curtains which, according to photometric measurements, reduced the amount of light to approximately one-half. Set "E" was placed in the same room and received full light.

The only variable which might then exist between Sets "D" and "E" besides that of light, is humidity and its effects on the soil moisture. The latter was adjusted, however, by means of atmometer readings (4). The variation in temperature was so slight that no provision was made for its control other than that of growing the sets over a long period of time.

As a source of additional irradiation the G. E. Sun Ray Lamp (S-I), (5) with a high transmission of visible and ultraviolet radiations in the regions of 3000 to 4000 Å was used. These irradiations when applied during the daytime were supplementary to daylight. It was equipped with a tray filter consisting of Red Purple Ultra glass No. 597 of 5 mm. thickness containing a 2-cm. layer of distilled water, fitted closely to the reflector of the lamp. This allows for a slight transmission of ultraviolet from about 3130 Å to a slight transmission of the violet line at 4050 Å with a high transmission, about 85%, at 3650 Å. There is also a slight amount of extreme red at 7500 Å, transmitted by the glass. Thus essentially only the long ultraviolet radiations reached the plants from the lamp.

The accompanying Fig. 1 indicates the percentage of transmission of the various wavelengths of light by this glass (6).

The raying distance was standardized at 23 cm. from the tops of the plants to the bottom of the filter.

The plants to be assayed were cut off just below the cotyledons. Only average plants were taken for assay. Ten grams were quickly weighed off very accurately in a weighing bottle which

was then placed in the drying oven at a temperature of 118° C. and dried for twenty-four hours. Upon reweighing the percentage of dry-weight was calculated. The remainder of the green material was immediately weighed, ground in a cold room to prevent the liberation of HCN and placed in the generator. The amount of green material assayed was the same for all sets. On the basis of the dry-weight percentage of each lot of material to be assayed, the actual dry-weight was calculated and this weight was used in arriving at the percentage of glucoside present.

The plants were ground and the grinder washed with 150 cc. of distilled water. This material, to which had been added two drops of caprylic alcohol to prevent frothing, was then placed in the generator which consisted of a 1000-cc. sidearm, Erlenmeyer flask, fitted with a gas-tight, motor-driven stirrer, having a $^1/_{32}$" bore to allow for aeration (7). A delivery tube leading from the sidearm conducted the gas into a train of three absorption bottles equipped with Folin's ammonia tubes and containing a 5% KOH solution (8). A stream of air was sucked through the whole apparatus, while the stirrer was turning, thus conducting the HCN, liberated by the action of the enzyme, linase (9) on the glucoside, into the absorption bottles where it formed a weak KCN solution. Aeration was continued for eighteen to twenty-five hours. Shortly before the end of the assay 15 cc. of 20 per cent HNO_3 was added to each generator to insure the elimination of all of the HCN. Aeration was resumed for $^1/_2$ hour after the addition of the acid.

The absorption bottles were emptied and rinsed into a 500-cc. wide-mouth Florence flask. To the flask was then added 10 drops of a 5% KI solution and the KCN titrated with one-hundredth normal silver nitrate solution in a manner similar to that reported by Bishop (10). This consisted in adding the silver nitrate solution dropwise from a burette into the flask through which a beam of light passes. As soon as all of the KCN has been converted into the $KAg(CN)_2$ complex, the AgI begins to precipitate, producing the Tyndall effect.

The percentage of HCN and glucoside was then determined by Roe's formula:

$$\frac{0.0005404 \times \text{cc. } 0.01 \text{ N. } AgNO_3 \times 100}{\text{Dry weight of sample taken}} = \% \text{ HCN} \qquad (8)$$

The percentage of HCN thus obtained was multiplied by ten to obtain the approximate percentage of glucoside present.

Several checks were made under identical conditions and upon identical material, *i. e.*, material from the same flat, and material from different flats. In every case the variation in duplicate samples is less then 0.05% of glucoside.

RESULTS.

In preliminary assays it was found that an average of 1.618 per cent of glucoside was in the leaves of plants 4" high against 0.5192 per cent in the stems of the same plants.

(*a*) *Moisture.*—The relationship between the low moisture condition "A," the high moisture condition "B" and control condition "C" is shown in TABLE II and in Fig. 2 in which the percentage of glucoside is plotted against the growth period in days.

TABLE II.—(*a*) MOISTURE, PERCENTAGE OF GLUCOSIDE END OF FIRST WEEK.

	Set.	Series I.	Series II.	Series III.	Av. %.
L. M.	"A"	1.2200	1.2500	1.2250	1.2320
H. M.	"B"	0.8720	0.8250	0.8570	0.8510
Con.	"C"	1.4170	1.2350	1.2470	1.2990
		End of Second Week.			
L. M.	"A"	0.7575	0.7610	0.7590	0.7575
H. M.	"B"	0.9234	0.9195	0.9290	0.9240
Con.	"C"	0.5803	0.5730	0.5600	0.5711
		End of Third Week.			
L. M.	"A"	0.5370	0.5430	0.5560	0.5453
H. M.	"B"	0.6185	0.6340	0.6250	0.6258
Con.	"C"	0.5870	0.5950	0.6080	0.5966

(*b*) *Light.*—In the experiments with normal light and its effects on the glucosidal content three conditions of the plants are compared. In Fig. 3 the relationship between the low light condition, "D," the high light condition, "E," and the control condition, "C," is shown. Here again the percentage of glucoside is plotted against the length of time in days. The numerical results are shown in TABLE III. The difference in the amounts of light received by Sets "E" and "C" is discussed in the part of this paper which deals with the methods.

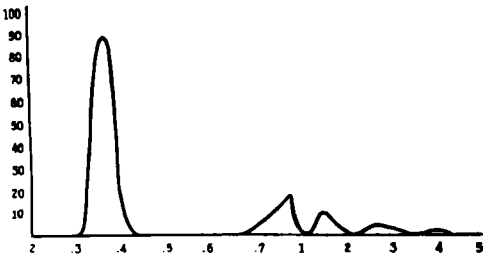

Fig. 1.—Transmission curve for Red Purple Ultra filter 5 mm. No. 597. The percentage of transmission is plotted against the wave-length.

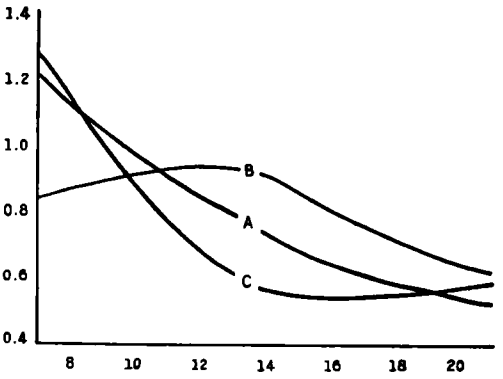

Fig. 2.—The effects of moisture variation on glucosidal content of flax. The percentage of glucoside is plotted against the time in days.

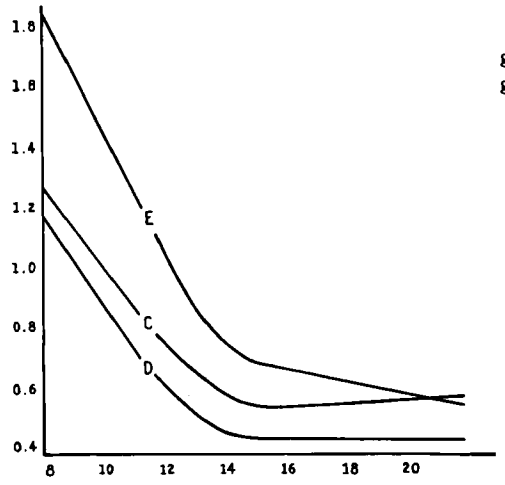

Fig. 3.—The effects of variations in light intensity on the glucosidal contents of flax. The percentage of glucoside is plotted against the length of time in days.

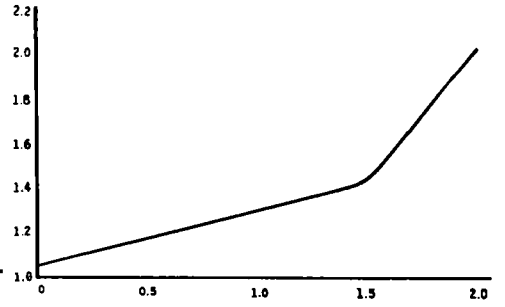

Fig. 4.—The rise in glucosidal content due to raying for various lengths of time. The percentage of glucoside is plotted against the raying time in hours.

TABLE III.—(*b*) NORMAL LIGHT, PERCENTAGE OF GLUCOSIDE END OF FIRST WEEK.

	Set.	Series I.	Series II.	Series III.	Av. %.
Con.	"C"	1.4170	1.2350	1.2470	1.2990
L. L.	"D"	1.1920	1.2320	1.2160	1.2133
H. L.	"E"	1.8700	1.9040	1.8380	1.8706
		End of Second Week.			
Con.	"C"	0.5803	0.5730	0.5600	0.5711
L. L.	"D"	0.4609	0.4813	1.4103	0.4508
H. L.	"E"	0.7102	0.7098	0.7123	0.7108

		End of Third Week.			
Con.	"C"	0.5870	0.5950	0.6080	0.5966
L. L.	"D"	0.4530	0.4740	0.4020	0.4430
H. L.	"E"	0.5710	0.5861	0.5790	0.5787

(*c*) *Artificial Light.*—The first experiment involving the use of artificial light, the results of which are given in TABLE IV, was undertaken to determine what possible effect raying, both during the daytime and at night, might have on the glucosidal content. It was also desired to see if any of the glucosidal material would disappear if the plants were kept in the dark for a number of days.

It was found that the flax plants which were rayed for two hours contained 2.62 per cent of glucoside on being assayed immediately after raying. A control set had 1.181 per cent of glucoside while the plants which were kept in the dark room contained only 0.684 per cent. The flat which was of the same age and grown under similar conditions to the three flats mentioned above was rayed for two hours during the night and assayed immediately after raying. The glucosidal content of this flat was 0.758 per cent.

The conditions of temperature and moisture were essentially the same throughout the experiment since the raying and assaying were performed within the same hour, in all of the flats except the one which was rayed at night.

TABLE IV.

Conditions of Plants.	Per Cent Glucoside.
Rayed for two hours during the daytime and assayed immediately after raying	2.62
Rayed the same night for two hours and assayed immediately after raying	0.758
Control set kept under the same conditions as set "C" described above and assayed at the same time as the first and fourth flats	1.181
Placed in the dark room and left there for two days. The plants were then cut in the dark room and assayed at the same time as the preceding flat	0.684

Considering the results obtained in the foregoing experiment with artificial light, it was thought advisable to see what effect several successive rayings would have upon the glucosidal content. For this experiment a single flat was used. One-half of it was rayed for two hours each night during the same number of nights. The plants were then allowed to remain in the greenhouse for twenty-four hours and were then assayed. It was found that very little difference in the glucosidal content resulted, the day-rayed plants containing 0.718 per cent while the night-rayed plants contained 0.688 per cent.

TABLE V.

Condition of Plants.	Per Cent Glucoside.
1/2 of a flat rayed two hours each day for 6 days. Assayed 24 hours after the last raying	0.718
The other half of the same flat rayed 2 hours each night for 6 nights. Assayed 18 hours after the last raying	0.688

Since the previous experiment undoubtedly indicated that the effect of raying on the glucosidal content of flax is somewhat temporary, from which the plant may recover if allowed to stand for a few hours, it became desirable to know what effect raying for various lengths of time would have on the glucosidal content. The curve representing the rise in glucosidal content with increased raying during the daytime, is indicated in Fig. 4. Here the plants were rayed for 0.5, 1.0, 1.5 and 2.0 hours and assayed immediately, beginning at 9 A.M. with the two-hour set and ending in the afternoon with the one-half hour set. The plants, although of different flats, were all of the same age (*i. e.*, twenty-one days old). They were grown under regular greenhouse conditions simulating those under which Set "C" was maintained. The raying time in hours is plotted against the percentage of glucoside found. TABLE VI serves as the basis for the graph.

TABLE VI.

Conditions of Plants.	Per Cent Glucoside.
No raying	1.050
Rayed for 0.5 hour	1.196
Rayed for 1.0 hour	1.300
Rayed for 1.5 hours	1.430
Rayed for 2.0 hours	2.030

Another experiment was carried out in which one flat, "C," grown under ordinary greenhouse conditions, and another flat, "A," grown under the same conditions but rayed for two hours, were cut immediately after the raying and the amount of glucoside hydrolysis determined at the end of 1, 2, 3, 4, 5, 18 and 25 hours. The results of this experiment are shown in TABLE VII. Here we see that the total glucoside formed in the rayed plants was 2.084% while that of the unrayed controls was 1.062%.

TABLE VII.

Assay Time in Hours.	% Glucoside Normal Set "C."	% Glucoside Rayed Set "A."
1	0.459	0.410
2	0.364	0.490
3	0.171	0.640
4	0.001	0.490
5	0.001	0.001
18	0.001	0.001
25	0.065	0.052
Total	1.062%	2.084%

DISCUSSION AND CONCLUSIONS.

The effect on the glucosidal content of flax produced by growth in a low moisture content soil, as can be seen in Fig. 2, has been generally that of reducing the amount of this substance. Although one point on the low moisture curve stands above that of the control set curve, it is believed that this difference is probably the exception rather than the rule. Flax plants, grown in a soil containing a high percentage of water, show a decided increase in the glucosidal content over the control condition. It is believed that at the time of the first assay on the plants, the effects of the various conditions had not yet become pronounced. It seems further possible that in the case of Set "B" the added water at first either caused an increased rate of translocation or increased the rate of secretion of enzyme as suggested by the work of Pickler (11), causing perhaps a subsequent decomposition of the glucoside.

Reynolds (12) has suggested that the glucoside in flax may possibly be produced largely in the actively growing cells and gives as evidence the fact that the percentage of glucoside decreases as the plants grow older and that the young tops of old flax plants contain almost as high a percentage of glucoside as do young plants. These facts, together with the fact that plants grown in a dry soil produce fewer new leaves, probably explain why the low soil-moisture set should give a low yield and the high soil-moisture set a high yield of glucoside. In the same paper Reynolds reported that healthy flax plants of a given age yielded 0.63% glucoside as against yellow plants (low soil-moisture growth) of the same age which yielded 0.083% glucoside. This certainly suggests that the percentage of moisture in the soil must affect the percentage of glucosides present in flax.

The reaction of the plants to a high light condition has been that of increasing the glucosidal content. In Fig. 3 we see that in the high light condition the plants show a high percentage of glucoside at the time of the first assay. This percentage decreases more or less regularly with the control "C." The fact that the low light curve "D," parallels the other two, suggests, no doubt, a relationship between these three conditions. Such results might be compared with the work of Eckerson (13) which suggests that low light conditions produce a retardation in the activity of reductase. If the glucoside-building mechanism, however, is stimulated by light, then we probably have an explanation for the results obtained under high light conditions

In the experiments in which artificial light was used it has been shown that long ultraviolet radiations are certainly efficacious in bringing about a variation in the glucosidal content of flax. In the plants rayed during the daytime the enzyme, linase, is probably stimulated in such a way as to build up more glucoside.

During the day a constant supply of carbohydrates plus the stimulation of nitrogen absorption, as demonstrated by Tottingham and Loswma (14), provide more favorable conditions for glucoside-synthesis than at night. During the night time ultraviolet light seems to invest the enzyme (linase) with added destructive powers. As for the constructive effects of ultraviolet light we have the work of Tottingham and Moore (15) who found a definite increase in protein, or in non-protein, non-lipide nitrogen in several economic plants under Vitaglass. There is also the work of Miss McCrea (2) which suggests a similar effect of light through Vitaglass on the seedlings of *Digitalis purpurea L.* The increase in glucosidal content reported by Miss McCrea, although contested by Arthur and Leonard (3) may be an actuality in view of the results reported here. The conditions of the drying process were not the same in the two experiments and it has been found by Hamilton (16) that great variations in chemical constitution exist in drugs under different conditions of light and temperature. Especially is this true of glucosidal drugs. There is also the factor of the time of harvesting, which, in the case of *Digitalis purpurea L.*, is a very important one. Miss McCrea collected her samples at the time of flowering which is accepted as the correct time for obtaining a high glucosidal-yielding drug. There was also the method of sampling to be considered. Arthur and Leonard used six plants from each group for analysis while Miss McCrea selected two samples from each group, each sample consisting of the leaves of 20 plants. In the latter experiment small differences of individual plants were ruled out because of the number of plants used in the assay.

It may well be that Miss McCrea's higher glucosidal values are associated with some such temporary factors as are demonstrated as important in the present work.

That the glucoside is decreased in quantity during the night raying and also during the two days in the dark room, a condition which would greatly reduce the labil carbohydrate content, is shown in TABLE IV. It was also found that the high or low glucosidal content of plants rayed over a period of days and nights, respectively, would, if the plants are allowed to recover for a few hours, return to a uniform percentage of glucoside. This suggests that the immediate effect of raying is a temporary one. These results are shown in TABLE V. In this connection it might be mentioned that Kerstan (17) in studying the physiological significance of the glucosides in several species of *Aesuclus* and *Salix* found an increase in the glucosidal content in the leaves during the daytime and a corresponding decrease at night-time in much the same way as the carbohydrates.

The quantitative effect of raying with ultraviolet is shown in TABLE VI. Here the percentage of glucoside seems to be proportionate to the length of raying time and probably indicates that the glucoside-producing mechanism is stimulated not only by the quality of light but also by the length of time it is applied. It is to be noted that in all of these ultraviolet experiments only the long ultraviolet irradiations from the lamp reached the plants.

The last experiment in which the HCN, calculated in terms of glucoside, liberated during 1, 2, 3, 4, 5, 18 and 25 hours from rayed and unrayed sets, was determined gives a further indication of the reactivity of the glucoside mechanism to ultraviolet irradiation. Although the hydrolysis took place somewhat more rapidly during the first hour from the unrayed set by the end of the third hour more HCN had been released from the rayed set and the total amount released was almost twice as much as from the unrayed set. The hydrolytic, as well as the synthetic reaction was stimulated by exposure of the plants to long wave ultraviolet.

SUMMARY.

(1) Several flats of flax were grown in a soil containing a low percentage of moisture. This condition produced a high dry-weight precentage but lowered the glucosidal content slightly from the control.

(2) Similar sets grown in a soil containing a high percentageof moisture showed a low dry-weight percentage and an increase in the percentage of glucoside over that of the control. This is possibly due to the fact that the glucoside-producing enzyme is secreted in greater quantities in plants under a high moisture condition.

There is also the fact that the glucoside is probably produced only in the actively functioning cells and since the leaves contain considerably more glucoside than that found in the stems and, furthermore, since the wet soil sets contained a considerably greater percentage of water, there was of necessity a greater number of actively functioning cells and consequently a higher percentage of glucoside.

(3) Plants grown in full light show a marked increase in the glucosidal content over those grown under low light conditions.

(4) Long ultraviolet radiations, when applied to plants during the daytime, invariably increased the percentage of glucoside. When applied at night the opposite or no effect was produced.

(*a*) The effect of the long ultraviolet, therefore, is apparently that of the activation of the glucoside-producing mechanism. This effect, however, is only a temporary one from which the plants may recover in a short period of time.

(*b*) It was further shown that the glucoside of flax largely disappears during forty-eight hours in the dark.

(*c*) The rate of decomposition of glucoside from rayed flax seems to differ somewhat from that of unrayed flax.

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"What is being done in hospital pharmacy to-day? Colleges of Pharmacy have recognized that real professional pharmacy is a necessary adjunct to every hospital and so have studied this problem. They also know that the real pharmacist must be trained in hospitals as well as in schools, and many schools have established this relationship and at the same time many hospitals have arranged internships for pharmacists. As a result, the American College of Surgeons in October 1936, adopted minimum standards for a hospital pharmacy. This will mean that soon every hospital must have a properly trained pharmacist and a well-equipped pharmacy, or secure pharmaceutical service from an *approved* pharmacy outside.

"The AMERICAN PHARMACEUTICAL ASSOCIATION has established a sub-section to study hospital needs from the pharmacist's standpoint, and the American Hospital Association has appointed a committee to study hospital pharmacy from the hospital's standpoint. These two committees are coöperating."—From Pharmacy Week Address of Edward Spease.
