acids have also been isolated by the procedure. An unknown was tentatively identified as a fructosan.

3. Sedoheptulose was translocated down the plant stalk, as were glucose, fructose, and sucrose.

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Literature Cited

1. Bennet-Clark, T. A. The rôle of organic acids in plant metabolism. Part II. New Phytol. 32: 128–230. 1933.
2. Proner, M. The presence of heptoses in some species of *Sedum* L. Bull. Sci. Pharmacol. 43: 7–14. 1936.
3. Nordal, A. and Klevstrand, R. Studies of the constituents of crassulacean plants. I. Paper chromatographic investigation of the free sugars of some *Sedum, Sempervivum, Echeveria,* and *Crassula* species. Acta Chem. Scand. 5: 85–88. 1951.
4. Nordal, A. and Öiseth, D. The occurrence of sedoheptulose in the dried root of *Primula elatior* (L.) Hill. Acta Chem. Scand. 5: 1289–1292. 1951.
5. Nordal, A. and Klevstrand, R. Studies of the constituents of crassulaceous plants. II. Paper chromatographic investigation of the free sugars of some *Rochea, Kalancoë, Cotyledon, Aeonium, Monanthes, Rosularia, Altamiranoa,* and *Echeveria* species. Acta Chem. Scand. 5: 888–890. 1951.
6. Nordal, A. and Öiseth, D. On the occurrence of sedoheptulose in certain species and genera of the plant family Saxifragaceae. Acta Chem. Scand. 6: 446–447. 1952.
7. Benson, A. A., Bassham, J. A., Calvin, M., Hall, A. G., Hirsch, H. E., Kawaguchi, S., Lynch, V., and Tolbert, N. E. The path of carbon in photosynthesis. XV. Ribulose and sedoheptulose. Jour. Biol. Chem. 196: 703–716. 1952.
8. Axelrod, B., Bandurski, R. S., Greiner, C. M., and Jang, R. The metabolism of hexose and pentose phosphates in higher plants. Jour. Biol. Chem. 202: 619–634. 1953.
9. Pratt, J. W., Richtmyer, N. K., and Hudson, C. S. Proof of the structure of sedoheptulosan as 2,7-anhydro-β-D-altroheptulopyranose. Jour. Amer. Chem. Soc. 74: 2200–2214. 1952.
10. Tolbert, N. E. and Zill, L. P. Metabolism of sedoheptulose-C^{14} in plant leaves. Arch. Biochem. Biophys. In press.
11. Burris, R. H., Wilson, P. W., and Stutz, R. E. Incorporation of isotopic carbon into compounds by biosynthesis. Bot. Gaz. 111: 63–69. 1949.
12. Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A., and Stepka, W. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. Jour. Amer. Chem. Soc. 72: 1710–1718. 1950.
13. Block, R. J., LeStrange, R., and Zweig, G. Paper Chromatography. Academic Press, Inc., New York. 1952.
14. Klevstrand, R. and Nordal, A. A spraying reagent for paper chromatograms which is apparently specific for ketoheptoses. Acta Chem. Scand. 4: 1320. 1950.
15. Denison, F. W. and Phares, E. F. Rapid method for paper chromatography of organic acids. Anal. Chem. 24: 1628–1629. 1952.
16. Putman, E. W. and Hassid, W. Z. Isolation and purification of radioactive sugars by means of paper chromatography. Jour. Biol. Chem. 196: 749–752. 1952.
17. Mueller, J. H. The use of thick paper for chromatography. Science 112: 405–406. 1950.
18. Anthony, D. S. and Long, M. V. Analyses for total and isotopic carbon in intermediary metabolism studies. Oak Ridge National Lab. Report No. 1303, Oct. 31, 1952. Unclassified.
19. Bassham, J. A., Benson, A. A., Kay, L. D., Harris, A. Z., Wilson, A. T., and Calvin, M. Path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor. Jour. Amer. Chem. Soc. 76: 1760–1770. 1954.
20. Zill, L. P., Khym, J. X., and Cheniae, G. M. Further studies on the separation of the borate complexes of sugars and related compounds by ion-exchange chromatography. Jour. Amer. Chem. Soc. 75: 1339–1342. 1953.
21. Vernon, L. P. and Aronoff, S. Metabolism of soybean leaves. IV. Translocation from soybean leaves. Arch. Biochem. Biophys. 36: 383–398. 1952.

THE EFFECT OF AUXIN ON THE FLOWERING BEHAVIOUR OF WINTEX BARLEY AND PETKUS RYE[1]

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Investigations of the problem of auxin in relation to flowering have so far been confined to experiments in which solutions of growth substances have been applied to plants grown under favourable or unfavourable photoperiods.

Auxin has been shown to inhibit flowering in a number of short-day plants: Dostal and Hosek (4) with Circaea; Bonner and Thurlow (1) with Xanthium; Harder and Van Senden (7) with Kalanchoë; Leopold and Thimann (8) with teosinte; and also in the long-day plant Calendula by von Denffer and Grundler (12).

It appears that the only clear-cut instance of flower promotion by applied auxin is in the pineapple

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(3, 13, 14). Small quantities of α-naphthaleneacetic acid or other active substances will induce inflorescence formation in plants that otherwise remain vegetative for several seasons. From the preliminary data of van Overbeek (14) it appears that the pineapple is a short-day plant.

Leopold and Thimann (8) describe the effects of α-naphthaleneacetic acid on the flowering behaviour of barley, variety Wintex, a long-day plant (2). Solutions of this auxin in low concentration in the order of 0.01 mg/l, when applied through cut leaves, increased the number of flower primordia on the developing ear over that of the controls. When the concentration was higher than 10 mg/l the number of flower primordia was decreased. These effects were accompanied by parallel changes in plant weight and axis length. Leopold and Thimann interpret these results as a " promotion " and " inhibition " of " flower initiation " respectively, taking the " relative abundance of flower primordia as a measure of flower initiation." From this and other observations they develop the hypothesis that in barley there is an optimal level of auxin for flowering, and that factors influencing the auxin level in the plant, will, under certain conditions, modify its flowering behaviour.

The experiments described in this paper were undertaken in order to confirm the effects of applied auxin on barley and to investigate the possibility of similar effects on rye.

Materials and Methods

Experiments with Petkus Rye: In 1950 a large scale experiment was carried out with rye, variety Petkus. The details of the experimental procedure followed that described by Leopold and Thimann for barley. Plants injected with auxin solution were treated as follows: the distal half of the blade of the youngest mature leaf on each plant was cut off with a razor and the remaining part of the lamina inserted in a small test tube containing one ml of auxin solution. The control plants were treated with water. A cotton wool plug was used to maintain the cut leaf in position and to reduce evaporation. The tubes were removed from the plants after five days by which time only 0.1 to 0.3 ml had been taken up by each plant (allowing for evaporation through the cotton wool plug). Under similar conditions it was found that aqueous dye solutions such as acid fuchsin or light green were rapidly absorbed into the vascular system, the whole plant being pervaded within a few hours. This absorption, however, represented a very small volume. Leopold and Thimann report that their solution (viz. 1 ml) was " generally entirely taken up by the plant within seven days."

Both the spring and winter varieties of Petkus rye were used. Experimental plants of the winter variety comprised two vernalised (10 weeks and 4 weeks at low temperature) and an unvernalised series. Vernalisation was carried out by the methods described by Purvis and Gregory (11). Each series was divided between long-days and short-days. The winter rye was first injected 3 weeks after the time of sowing and further injections were carried out after successive intervals of three weeks as long as the plants remained in a vegetative condition. All plants that had not reached the stage of ear-emergence 19 weeks from sowing were dissected after this time.

The spring rye was grown for 3 weeks in short-days; it was then injected and transferred to long-days. These plants were dissected after a further 3 weeks, that is, when they were 6 weeks old.

The plants were grown in sand culture in 10-inch pots with nutrients as described by Purvis and Gregory (10). Short-days (10 hours) were provided by the use of light-proof covers; long-days by the extension of the natural daylength when necessary to 17 hours by artificial light of low intensity (20 fc).

Experiments with Wintex Barley: In 1951 and in the following two years, experiments were carried out with barley, variety Wintex. The experimental procedure followed exactly that described above for spring rye: seedlings were first grown for 3 weeks in short days. Dissection of plants after this time revealed that they were in a vegetative condition. They were then transferred to 17-hour days and injected with auxin solution. Five days after injection, 0.2 to 0.5 ml of solution had been absorbed by each plant. It will be noted that the absorption rate was higher in the barley than in the rye. The plants were dissected when they were 6 weeks old, that is 3 weeks from the time of injection.

Results

Experiments with Petkus Rye: Two concentrations of α-naphthaleneacetic acid were tested, a low concentration (0.01 mg/l) and a high concentration (30 mg/l). Neither of these concentrations influenced the time to flowering, the number of flower primordia, the leaf number, or the rate of tillering for each series of plants. No data for these experiments will therefore be presented.

Experiments with Wintex Barley: The results of two experiments with Wintex barley carried out in the summer of 1951 are presented in Table I. Replication in the first experiment was 15 plants and in the second experiment 10 plants per treatment. Variability is expressed as the standard error of the mean. The auxin solutions tested were from a series of dilutions commencing with a saturated aqueous solution of α-naphthaleneacetic acid at 20°C which is approximately 500 mg/l. The figures given in the table for the concentrations of auxin are therefore approximate.

Flower primordia are defined here as the initial or ridge at each node that gives rise to a group of 3 spikelets. Counts of flower primordia were confined to the main axis of each plant. Every concentration of auxin with the exception of the highest used (approximately 500 mg/l) increased the number of flower primordia over that of the controls. Some of the differences are statistically significant with a value of P lower than 0.01. The highest concentration reduced the number of flower primordia in the second

TABLE I

THE EFFECT OF INJECTED AUXIN (α-NAPHTHALENEACETIC ACID) ON THE NUMBER OF FLOWER PRIMORDIA, SPIKE LENGTH, AND TILLERING OF WINTEX BARLEY

CONCENTRATION OF NAA (APPROX. MG/L)	NUMBER OF FLOWER PRIMORDIA PER PLANT	SPIKE LENGTH (SCALE UNITS)	NUMBER OF TILLERS PER PLANT
Sowing Date: 30/5/51 Replication 15			
500	22.3 ± .39	56.1 ± 3.4	6.5 ± .59
0.5	25.6 ± .61	104.5 ± 8.3	6.9 ± .39
0.1	24.4 ± .59	91.1 ± 8.7	6.5 ± .24
0.05	23.3 ± .51	80.9 ± 4.3	7.1 ± .22
0.01	23.3 ± .60	84.6 ± 5.7	6.4 ± .24
Control	21.9 ± .46	64.5 ± 3.2	6.0 ± .41
Sowing Date: 17/6/51 Replication 10			
500	22.6 ± .86	32.4 ± 2.7	5.4 ± .82
50	30.1 ± .61	46.4 ± 2.0	5.9 ± .82
5	29.5 ± .65	42.1 ± 2.1	5.4 ± .48
0.5	31.2 ± .42	46.1 ± 1.9	7.5 ± .40
0.05	29.8 ± .71	43.7 ± 2.2	6.0 ± .52
0.005	29.1 ± .48	44.2 ± 1.1	6.2 ± .42
Control	26.7 ± .40	39.0 ± 2.0	4.7 ± .52

experiment but not in the first. These changes were parallelled by similar changes in the length of the spike. Tillering was slightly increased by all concentrations.

In the experiments of Leopold and Thimann the changes in number of flower primordia were exactly parallelled by changes in fresh weight. In the experiments recorded here, similar but slight changes in fresh weight were found but none of the differences were statistically significant.

The increase in number of flower primordia following injection of auxin in low concentration is thus confirmed, although the magnitude of this and corresponding effects was much less than that obtained by Leopold and Thimann. The increase in number of flower primordia in the experiments recorded here averaged 10 to 15 % of the controls, and the decrease by the high concentration in the second experiment approximately 15 %. Leopold and Thimann obtained a 40 % increase with a concentration of 0.01 mg/l, while the reduction in number of primordia by their highest concentration (400 mg/l) approached 80 %.

Figure 1 is a photograph of the young ears that were dissected out of the plants that were used in the first experiment of 1951 (sowing date: 30/5/51). It can be seen that the ears from plants injected with low concentrations of auxin, especially concentrations of 0.1 and 0.5 mg/l, are at a more advanced stage in spikelet differentiation. The figures on the right of figure 1 show the relative sizes of the primordia as calculated by dividing the length of the ear by the number of initials present. These differences may be attributed to (a) earlier initiation of flower primordia and/or (b) a post-initiation increase in the rate of development of the spike. It must be emphasised that the ear at this stage has entered upon the exponential phase of the grand period of growth, so that if the observed differences were due entirely to earlier initiation, an acceleration of 2 or 3 days would fully account for the larger ears in the treated plants from this experiment.

In February of 1952 and in the same month of 1953 the experiments described above were repeated on a larger scale with a view to ascertaining the time of initiation of flower primordia by the dissection of periodical samples. In this way it could be decided whether auxin treatment either hastened initiation or merely stimulated the post-initiation development of the spike. In these repeated tests the solution was absorbed to the extent of 0.3 to 0.5 ml per plant in 5 days. The lower concentrations employed failed to increase the number of primordia but the high concentration reduced the number of primordia by 15 %. In all treatments flower primordia were initiated on the spike within a week after transfer to long-days. As the results of these two experiments failed to show any stimulating effect on either initiation or primordia number no data will be presented.

DISCUSSION

In considering the possible effects of auxin on flowering behaviour it is necessary to define the meaning in which " promotion of flowering " or " favourable to flowering " are used. The changeover from the vegetative to the reproductive phase is unequivocally indicated by the first appearance of flower primordia, and this critical stage in development is denoted by the term " initiation." The interval elapsing between the beginning of treatment and this point in development can be measured either in days or in terms of the plastochrone (leaf number). The advantage of the latter measure accrues from the fact that it is independent of the rate of differentiation at the growing point; thus the same number of plastochrones may demand different periods of time at different temperatures.

Conditions of " promotion of flowering " or " favourable to flowering " may on the other hand be assessed by the number of flower primordia laid down and it would appear that Leopold and Thimann have used this as a measure of the intensity of flower induction.

In the Gramineae the number of flowering ridges developed on the ear is not directly associated with flower initiation, nor is this number uniquely determined by the photoperiodic conditions. A positive correlation is found between spikelet number and the weight of the plant (as Leopold and Thimann have shown in barley) and a similar correlation is found between spikelet number and the age of the plants when flowers are initiated (2, 5, 6, 9). Increasing the level of nitrogen supplied in the nutrients results in larger and heavier plants with more numerous flower primordia, while the time to flower initiation is unaffected (5, 9). The time to initiation depends only on the conditions of light and temperature, while the

number of spikelets is influenced by these factors only indirectly (that is, only in so far as light and temperature determine the onset of the reproductive phase).

In view of this discussion, it is clear that the ferred to long-days when they were injected with auxin (that is injected and maintained in the former regime of short-days) the plants were still vegetative at the time of dissection. Auxin alone therefore does not hasten flower initiation in barley; at best it might

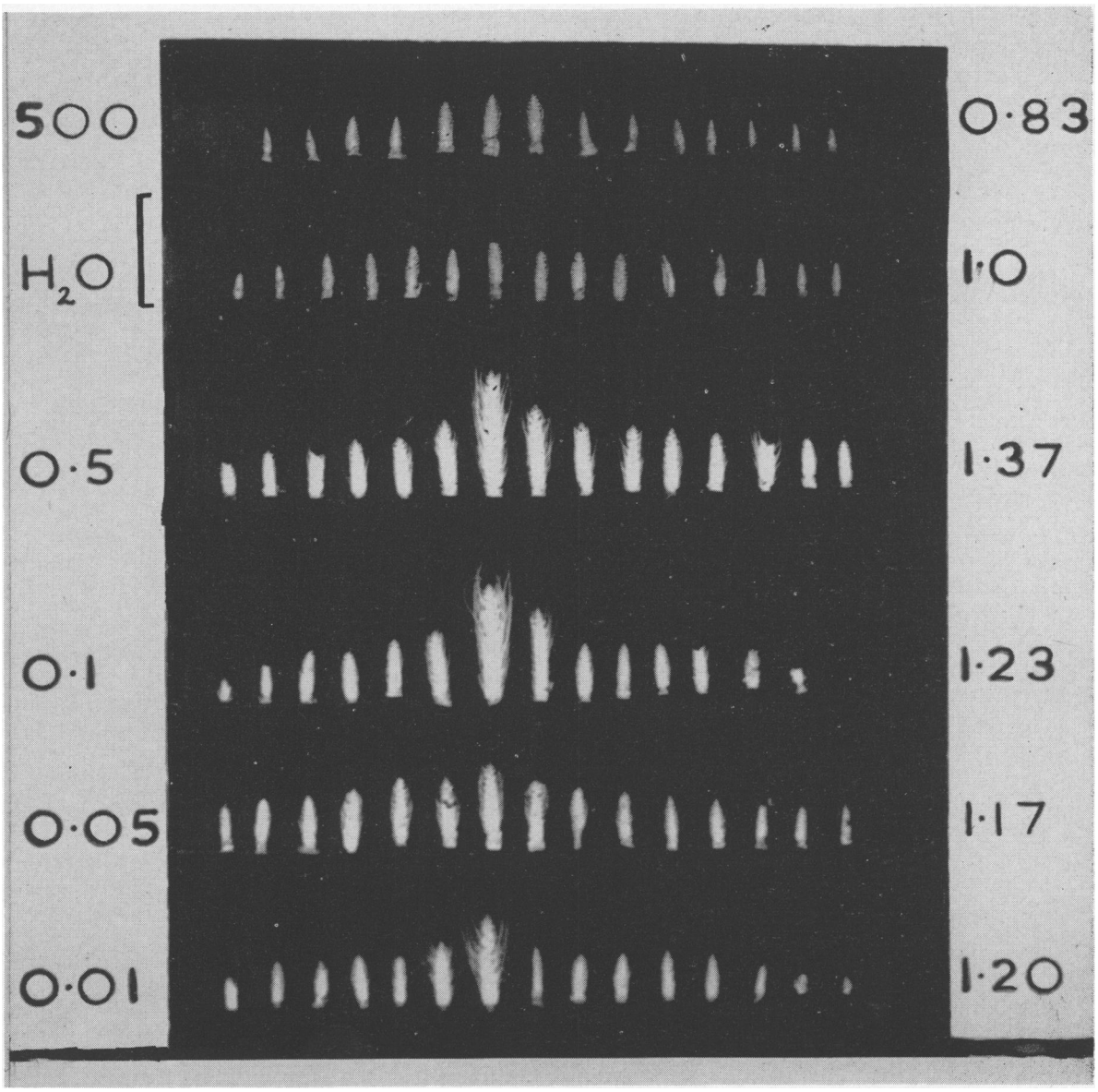

FIG. 1. Photograph of the young ears that were dissected from the Wintex barley plants used in the first experiment carried out in 1951, sowing date: 30/5/51. The figures on the left give the concentration of auxin solution injected (see table) and the figures on the right the relative sizes of the flower primordia. The scale printed on the left of the water controls equals 1 cm.

"relative abundance of flower primordia" does not provide a "measure of flower initiation" in barley, but may indicate an effect of auxin on post-initiation development of the ear. An effect on initiation could only be claimed if injected auxin caused initiation to occur in unfavourable photoperiods. Leopold and Thimann showed that if the plants were not transenable the plants to respond more readily to long photoperiods. But since after transfer to long photoperiods, initiation is manifest within a week when no auxin is applied, any enhancement in the response brought about by auxin can only be small, at the most a few days acceleration. Such a slight improvement is suggested from the results of the 1951 experi-

ments reported here, but as already pointed out, these results could also be accounted for on the basis of a purely post-initiation effect. In two experiments out of four carried out with Wintex barley no stimulating effects of auxin were found.

Summary

Plants of Petkus rye and Wintex barley were injected with auxin (α-naphthaleneacetic acid) according to the methods used by Leopold and Thimann for Wintex barley. The flowering behaviour of Petkus rye was not affected by the auxin. In two experiments out of four carried out with Wintex barley, auxin in low concentration increased the number of flower primordia while high concentrations decreased the number of primordia. This result, obtained in two experiments, confirms the findings of Leopold and Thimann, although the magnitude of the effects was much less in the experiments reported here.

It is suggested that the results of Leopold and Thimann may be evidence for a post-initiation effect of auxin on the developing ear, and in this sense may be said to promote or inhibit flowering, but there is no evidence either in the experiments of Leopold and Thimann or those reported here, that auxin has any determinable effect on flower initiation.

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Literature Cited

1. Bonner, J. and Thurlow, J. Inhibition of photoperiodic induction in Xanthium by applied auxin. Bot. Gaz. 110: 613–624. 1949.
2. Borthwick, H. A., Parker, M. W., and Heinze, P. H. Effect of photoperiod and temperature on the development of barley. Bot. Gaz. 103: 326–341. 1941.
3. Clark, H. E. and Kerns, K. R. Control of flowering with phytohormones. Science 95: 536–537. 1942.
4. Dostal, R. and Hosek, M. Über den Einfluss von Heteroauxin auf die Morphogenese bei Circaea. Flora 139: 263. 1937.
5. Gott, M. B. The response to photoperiod and nitrogen nutrition in the vernalisation of Petkus rye. Ph.D. Thesis, London University. 1950.
6. Hänsel, H. Über die Wirkung des Kurztages auf Zeit des Aehrenschiebens und auf Aerchenzahl pro Aehre bei Petkus Winterroggen. Die Bodenkultur 5: 305–312. 1951.
7. Harder, R. and Van Senden, H. Antagonistische Wirkung von Wuchstoff und "Bluhhormon." Naturwiss. 36: 348. 1949.
8. Leopold, A. C. and Thimann, K. V. The effect of auxin on flower initiation. Amer. Jour. Bot. 36: 342–347. 1949.
9. Purvis, O. N. An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. 48: 919–955. 1934.
10. Purvis, O. N. and Gregory, F. G. Studies in the vernalisation of cereals. I. A comparative study of vernalisation of winter rye by low temperature and by short-days. Ann. Bot. N.S. 1: 569–592. 1937.
11. Purvis, O. N. and Gregory, F. G. Studies in vernalisation. XII. The reversibility by high temperature of the vernalised condition in Petkus winter rye. Ann. Bot. N.S. 16: 1–21. 1952.
12. von Denffer, D. and Grundler, H. Über Wuchsstoffinduzierte Blühhemmung bei Langtagpflanzen. Biol. Zentr. 69: 272–282. 1950.
13. van Overbeek, J. Flower formation in the pineapple as controlled by 2,4-D. Science 102: 621. 1945.
14. van Overbeek, J. Control of flower formation and fruit size in the pineapple. Bot. Gaz. 108: 64–73. 1946.