



Suppressive Effects of 2-Thiouracil on Differentiation and Flowering in *Cannabis sativa*

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Source: *Science*, New Series, Vol. 132, No. 3444 (Dec. 30, 1960), pp. 1943-1944

Published by: American Association for the Advancement of Science

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[International Union of Geodesy and Geophysics (IUGG)], and J. F. Denisse [International Astronomical Union (IAU)], with R. Coutrez (URSI) as secretary. The IWDS obtains nominal support from the ICSU Federation of Astronomical and Geophysical Services. Shapley serves as spokesman for IWDS and as its correspondent to other ICSU groups such as the International Committee on Geophysics (CIG) and the Committee on Space Research (COSPAR).

The International Geophysical Calendar for 1961 was drawn up by Shapley and J. V. Lincoln in consultation with URSI, IUGG, and IAU, both directly and through CIG and COSPAR. Recommendations also have come from representatives of WMO and from interested individual scientists. A similar calendar was issued for 1960 along the lines of the calendars for the IGY and International Geophysical Cooperation 1959, issued under the auspices of the ICSU Special Committee for the International Geophysical Year and described in the "IGY Instruction Manual for World Days and Communications" (3).

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References and Notes

1. Copies of the calendar are available upon request to the Secretary General, International Scientific Radio Union, 7 Place Emile Dancu, Brussels 18, Belgium.
2. More detailed recommendations by working group 2 of the Committee on Space Research (J. Bartels, chairman) appear in the *COSPAR Information Bulletin*.
3. *IGY Annals* (Pergamon, New York, 1959), vol. 7.

25 November 1960

Suppressive Effects of 2-Thiouracil on Differentiation and Flowering in *Cannabis sativa*

Abstract. The pyrimidine, 2-thiouracil, partly annuls the effect of photoperiodic induction in the short-day plant, *Cannabis sativa* L., when it is supplied at the onset of the dark period in quantities of 15–30 μg per plant. This treatment also produces aberrations in cellular differentiation in the leaves. Tracer studies show that 2-thiouracil becomes bound in cellular ribonucleic acid, which suggests that the effects on morphogenesis are due to interference with nucleic acid metabolism.

2-Thiouracil is highly effective in suppressing or retarding certain processes of development and differentiation in the short-day plant *Cannabis sativa* (hemp) in concentrations which do not immediately inhibit normal cell extension growth. In the experiments in which these effects have been observed, hemp plants were grown in growth

rooms in light at 900 to 1000 ft-ca, with air temperature regulated at 22°C and relative humidity at 70 percent. The plants were kept in a vegetative condition in a daylength of 18 hours to ages of 3 to 4 weeks, after which photoperiodic induction was given by transfer to a daylength of 8 hours. The 2-thiouracil was supplied by painting standard areas of the upper epidermis of the youngest fully expanded leaves with an aqueous solution containing 100 $\mu\text{g}/\text{ml}$, the area being adjusted to establish the required dosage. Traces of detergent ("Teepol") were added to aid wetting.

In hemp, a dioecious species, exposure to seven or more short days at an age of 3 weeks induces the formation of fertile flowers at several successive nodes of the main stem in both sexes (1). Daily dosages of 2-thiouracil in the range of 15 to 30 μg per plant, supplied just before the onset of the dark period, severely reduced the flowering response of male plants to an induction period of 10 short days, and almost abolished the response of female plants. In one experiment, short-day induction began at an age of 25 days with batches of eight plants, the first 2-thiouracil treatment being given just before transfer to the first dark period. In the control plants of genetically male sex, the first fertile flowers were differentiated uniformly at the sixth node of the main stem; in male plants receiving 2-thiouracil, the first distinguishable buds appeared at node 8, but these were always without anthers. Among the female plants, the mean node number for the first fertile flowers in the controls was 7; of the plants of this sex receiving 2-thiouracil, only one formed a distinguishable bud during the 4 weeks following induction, and this, at the ninth node, was without gynoeceum.

In this experiment plants of both sexes showed some retardation in general development as a result of treatment during photoperiodic induction. Although in part the reduction in flowering response may be attributable to this, it seems likely that the effect is more specific since there was little flowering during the recovery period, and the plants ultimately resumed vegetative growth. This suggests a partial blocking of the developmental processes involved in flowering, and it is significant that in some of these experiments it was accompanied by histological aberrations suggesting a parallel effect on cellular differentiation. Thus, with daily dosages of 10 to 30 μg of 2-thiouracil, striking modifications of leaf structure were initiated. During protracted periods of treatment, abnormalities became progressively more severe from node to node, leaves showing first a re-

duction of pigmentation attributable to a diminution of chloroplast population, then modifications in mesophyll structure, then a failure of the lamina to expand normally, accompanied by abnormalities in epidermal features such as stomata, and finally aberrations in vasculature.

This increasing severity of symptoms from node to node indicates a relationship with the age of leaf primordia, and the readiest interpretation of the sequence of effects is that it represents in inverse order that order in which the processes of cellular differentiation normally do occur in the leaf. The remarkable extent to which mesophyll differentiation is affected can be seen in Fig. 1, in which sections of the lamina of a normal leaf and that of one developed in the presence of 2-thiouracil two nodes above that of treatment are compared. In the modified leaf, while cellular growth has hardly been impaired, extension has been practically isodiametric in the cells of all layers, so that at their final volume the cells differ greatly in shape from their homologues in the normal leaf.

The fate of 2-C^{14} -2-thiouracil introduced through the leaf was followed autoradiographically. In one experiment, three doses were given at daily intervals. The upper leaves were then removed from one sample of plants to observe the immediate fate of the tracer, while another sample was permitted to grow on for 3 weeks to determine the final distribution. From autoradiographs prepared in the usual manner on Ilford x-ray film, it was found that the labeled 2-thiouracil became rapidly distributed in all growing tissues above the level of application, the accumulation in young leaf primordia being especially marked. After this early incorporation there was little subsequent redistribution, for the bulk of the tracer remained bound in the tissues which were actively growing at the time of treatment.

The chemical distribution of the bound 2-C^{14} -2-thiouracil was examined to determine what proportion could be associated with ribonucleic acid. The leaves were killed and thoroughly extracted with boiling 70 percent ethanol, and then defatted in ether. Samples of the alcohol and ether extracts were plated for counting. The leaves were then dried, pulverized, and the thoroughly homogenized powder was divided into two equal portions. One portion was suspended in distilled water, and the other in 0.01 percent ribonuclease (crystalline analytical grade, Biochemica Boehringer), and both samples were incubated overnight at 37°C. The undigested residues were centrifuged, and samples were taken of the supernatants for counting. The

extraction of the remaining nucleic acids was completed with normal perchloric acid at 70°C. Of the total amount of recovered radioactivity, 11 percent was present in the alcohol extract, and a negligible amount in the ether fraction. In the respective samples, ribonuclease digestion released 76 percent, and the water extraction, 32 percent, the balance in each case being located in the perchloric acid extract. This result suggests that the bulk of the radioactivity bound in these leaves was present in the ribonucleic acid fraction.

Various analogues of naturally occurring purines and pyrimidines have

recently been shown to have inhibitory effects on development and cellular differentiation in higher plants. Thus 5-fluorouracil partly annuls the effects of short-day induction in *Xanthium pennsylvanicum* (2), and 2-thiouracil similarly blocks flowering in *Streptocarpus wendlandii* (3). A remarkable case of the blockage of a developmental step on a cellular level has been reported for *Dryopteris erythrosa* (4), in which the transition from one- to two-dimensional growth in the gametophyte is blocked by 8-azaguanine. In all these cases, the implication is that the analogue functions by interfering with nucleic acid metabolism.

Salisbury and Bonner (2) suggest that, in the inhibition of flowering in *Xanthium*, 5-fluorouracil affects the synthesis or effectiveness of the products of the inductive dark period. However, it seems to me that the response is more likely to be due to a loss of capacity of apical meristematic tissues to react to leaf-generated stimuli. Viewed in terms of gene action, it may well be that the loci, quiescent during vegetative growth, which are activated following photoperiodic induction, are unable to produce their characteristic proteins in the presence of factors modifying intermediate stages of ribonucleic acid synthesis, so that the new developmental pathway, namely flowering, cannot be entered. The concomitant effects of 2-thiouracil on cellular differentiation described above could be taken to support such a hypothesis (5).

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References and Notes

1. J. Heslop-Harrison and Y. Heslop-Harrison, *Proc. Roy. Irish Acad. B* 59, 257 (1958).
2. F. B. Salisbury and J. Bonner, *Plant Physiol.* 35, 173 (1960).
3. D. Hess, *Planta* 54, 74 (1959).
4. Y. Hotta and S. Osawa, *Exptl. Cell Research* 15, 85 (1957).
5. A full account of this work will be given elsewhere. The experiments were performed with the facilities of the Queen's University, Belfast, to which institution I tender my thanks.

15 August 1960

Learned Behavior of Rhesus Monkeys following Neonatal Bilateral Prefrontal Lobotomy

Abstract. Bilateral ablation of dorsolateral frontal cortex in the newborn animal failed to produce the frontal lobe syndrome typical of the adolescent and adult stages. The crucial tests (delayed response, string tests, and discrimination learning) were performed 3 to 4 months postoperatively. At this time, no significant differences between normal animals and animals with prefrontal lesions were found.

The classical work of Jacobsen (1) demonstrated that adolescent and adult rhesus monkeys with bilateral lesions in the prefrontal areas show a severe deficit on the delayed-response problem and no appreciable loss in discrimination learning. This finding has been confirmed by many independent investigators, including Harlow *et al.* (2) and Pribram *et al.* (3). Furthermore, research workers in the area agree that there is no spontaneous remission of this syndrome with time. Recent researches reported by Harlow (4) demonstrate that the solution of delayed response by monkeys is a function of age and that this capability appears at about 125 days. The purpose of the study re-

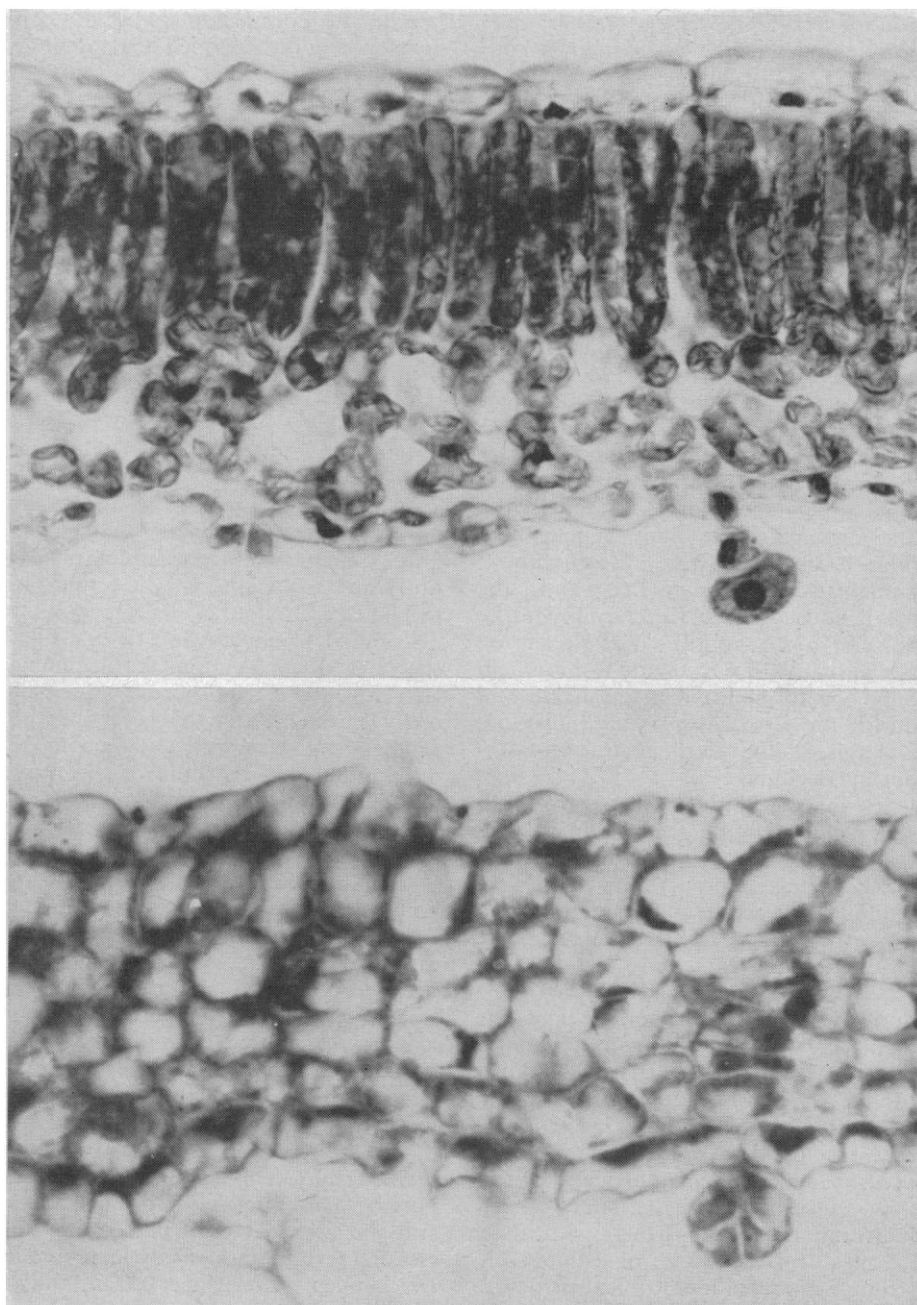


Fig. 1. (Top), Transverse section of the lamina of a normal hemp leaf, showing the characteristic differentiation of the mesophyll tissues. (Bottom), Section of a leaf of hemp developed in the presence of 2-thiouracil. The lamina is composed of a plate of undifferentiated parenchyma (about $\times 820$).