# A NOTE ON THE X-IRRADIATION OF SEEDS OF *DATURA TATULA* WITH SPECIAL REFERENCE TO ALKALOID PRODUCTION

By W. C. Evans and Maria J. Menéndez

*From The University, Nottingham*

Received December 2, 1955

The specific effects of X-rays on alkaloid production in plants appear to have received little attention. That radiations produce fragmentations, translocations and segmental interchange as well as gene mutation in *Datura stramonium* has been known for a considerable time and utilised for the isolation of prime types in this genus[1]. As an attempt to induce in *D. tatula* var. *inermis* mutations which might be of value in the study of alkaloid biogenesis the following experiments were performed.

TABLE I

Plants from irradiated seeds

| Plant | General Morphology | Hyoscine : Hyoscyamine per cent. dried plant |
|---|---|---|
| 1 | Small and retarded with one immature fruit. Leaf abscission occurred early and plant not collected .. .. .. | |
| 2 | Resembled controls .. .. .. .. .. .. | 0·06 : 0·20 |
| 3 | Somewhat contorted but generally resembled controls .. | 0·02 : 0·12 |
| 4 | Stunted in early growth, branching not typically dichasial, one shoot tending towards excessive development at the expense of the other. Lower leaves often incomplete. The first four flowers and fruits produced showed six divisions to their structure, the remainder four .. .. | 0·07 : 0·19 |
| 5 | Resembled Plant 4 but no mature fruits produced. Two immature fruits possessed 6 carpels with a few immature seeds .. .. .. .. .. .. .. | 0·06 : 0·23 |
| 6 | A stunted plant which produced no really mature fruits .. | 0·04 : 0·27 |
| 7 | Resembled controls .. .. .. .. .. .. | 0·05 : 0·30 |

Four groups, each of 50 moistened dormant seeds were subjected to X-ray doses of 5000, 10,000, 15,000 and 20,000 r respectively and the same day sown in seed compost, together with untreated seeds as controls. In those groups receiving 10,000 and 20,000 r, no seeds germinated and in the 15,000 r group one seedling developed but did not survive. In the 5000 r group many seeds germinated over a prolonged period. Most died before the cotyledons had fully expanded and others were chlorophyll deficient or badly mottled and gradually died. Seven seedlings survived, and when sufficiently large, were transplanted into open ground. Each plant was harvested at the flowering and fruiting stage and all excepting the seeds and roots analysed for hyoscine and hyoscyamine by the method of Evans and Partridge[2]. The separated alkaloids were in each case characterised by measurements of the optical rotations of the sulphate titration liquors, by the $R_F$ values of paper chromatograms and by the melting points and mixed melting points of the picrates with authentic compounds. The observations on these parent plants are summarised in Table I.

In the subsequent year, the first generation of the above plants was raised together with control plants. Only two seedlings could be raised from the largely immature seeds of Plants 5 and 6 (Table I). Few seeds of Plant 7 were available but their germination was good and numerous seedlings of Plants 2, 3 and 4 were obtained. At the flowering stage the plants were harvested and where available, twenty from each parent were tested for hyoscine and hyoscyamine by paper chromatography. A further selection were analysed quantitatively, the separated alkaloids being identified by the melting points of their picrates. The results are recorded in Table II.

TABLE II

PLANTS OF SECOND GENERATION

| Parent plant | General morphology of $X_1$ plants | Paper chromatography of extracts of $X_2$ plants | Hyoscine : Hyoscyamine as per cent. dried $X_2$ plants |
|---|---|---|---|
| 2 | 33 plants; all resembled controls | 20 plants tested. 19 contained both hysocine and hyoscyamine and one a trace of hyoscine only. With one exception the hyoscyamine content appeared below average | 0·06 : 0·09; 0·03 : 0·08; 0·04 : 0·07; 0·06 : 0·09; 0·08 : 0·11 |
| 3 | 44 plants; two showed abnormal branching and the remainder resembled the controls | 20 plants tested. All contained hyoscine and hyoscyamine in proportions in keeping with the quantitative estimations except for three instances in which the hyoscyamine content was very low | 0·03 : 0·09; 0·07 : 0·25; 0·1 : 0·15; 0·05 : 0·11; 0·01 : 0·09; 0·06 : 0·11 |
| 4 | 54 plants; all more or less resembled the controls except for three with abnormal branching | 20 plants tested. All contained hyoscine and hyoscyamine in apparently normal proportions | 0·06 : 0·10; 0·01 : 0·05; 0·13 : 0·21; 0·04 : 0·14; 0·07 : 0·12; 0·04 : 0·09; 0·05 : 0·14; 0·08 : 0·19; 0·04 : 0·12; 0·06 : 0·03; 0·04 : 0·10; 0·02 : 0·03; 0·04 : 0·10 |
| 5 | One plant; resembled the controls | | 0·006 : 0·01 |
| 6 | One plant; very small but of normal appearance | | 0·0 : 0·006 |
| 7 | 10 plants; all shorter than the controls with a denser foliage | Four plants tested. All appeared to contain more alkaloids than the controls | 0·03 : 0·28; 0·004 : 0·25; 0·07 : 0·18; 0·04 : 0·28; 0·04 : 0·24; 0·003 : 0·09 |
| | Control plants. Hyoscine : hyoscyamine As per cent. dried plants | 0·07 : 0·14; 0·07 : 0·11; 0·02 : 0·12; 0·08 : 0·13; 0·06 : 0·15; 0·03 : 0·15; 0·06 : 0·10; 0·04 : 0·13; 0·06 : 0·18; 0·05 : 0·18; 0·02 : 0·08; 0·03 : 0·12; 0·02 : 0·09; 0·02 : 0·08. | |

It is apparent that irradiation of seeds with 5000 r of X-rays produced mostly lethal effects of the types recorded with other plants. Among the surviving plants distinct morphological differences were often obvious although the nature of the alkaloids was unchanged. The total alkaloidal content of these plants exceeded that of the controls but this may have been without significance since the two groups matured under different environmental conditions due to their different rates of growth.

Similarly in the second generation, no new alkaloids were encountered although in isolated instances only one alkaloid could be detected. The variations in the relative proportions of the individual alkaloids were

not however, without interest. All the $X_2$ offspring from Plant 7, with one exception, gave relatively high hyoscyamine yields, those from Plant 2 possessed average quantities of hyoscine but consistently low amounts of hyoscyamine and those from Plants 3 and 4 contained varied total alkaloid contents with hyoscine : hyoscyamine ratios in most cases similar to the controls.

Such results have obvious implications in the selective breeding of desirable strains of *D. tatula* but further investigation would be required to establish whether variations of the type reported here are significantly different to those which have been isolated by conventional breeding methods[3,4]. It is probable that in the present instances, no mutations directly involving alkaloid biogenesis were involved but since alkaloid content and ratios of individual alkaloids are known to vary throughout the life-cycle of *D. tatula*[5], mutations involving many factors may indirectly affect the ultimate alkaloidal content.

We are indebted to Dr. M. W. Partridge for his help and advice and to Dr. F. H. Cross for arranging the radiation of the seeds at the General Hospital, Nottingham.

## References

1. Blakeslee, *Int. Conf. Genet.*, 1932, No. **6**, 104.
2. Evans and Partridge, *J. Pharm. Pharmacol.*, 1952, **4**, 769.
3. Miller and Meader, *J. industr. Eng. Chem.*, 1913, **5**, 1014.
4. Hegnauer and Flück, *Pharm. Acta Helvet.*, 1949, **24**, 1.
5. Evans and Partridge, *J. Pharm. Pharmacol.*, 1953, **5**, 772.