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The Potato Eelworm Hatching Factor

4. *SOLANUM NIGRUM* AS A SOURCE OF THE POTATO EELWORM HATCHING FACTOR

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It has been known for some time that the root excretion of several species of *Solanum* stimulates the hatching of larvae from the cysts of the potato eelworm, *Heterodera rostochiensis* (Franklin, 1940). The isolation of the active principle in root excretion and the investigation of its chemical nature was first attempted, using the leachings from potato plants (Hurst, 1935, 1937), but the method of production was cumbersome and a more convenient source of material was found in tomato plants. The tomato plants were more easily grown indoors than potatoes, and gave a very active excretion. As work on the chemical nature of the active principle developed (Calam, Raistrick & Todd, 1949; Marrian, Russell, Todd & Waring, 1949), greater demands were made upon production of starting material for the investigation, and necessitated the growing of many thousands of tomato plants during a season. It became clear that if the scale of the work continued to expand a new source of root excretion, which could be easily and cheaply grown out of doors on a large scale, would be welcomed to replace the growing of tomato plants under glass. With this end in view, a small scale outdoor experiment with *Solanum nigrum* was carried out during 1943 at the suggestion of Sir E. J. Salisbury, F.R.S., who also very kindly supplied the seeds, and the yield, quality and nature of the root excretion were examined.

METHODS

The growing and leaching of Solanum nigrum plants

The plants were grown from seed in two trays *A* and *B* (each 4 × 3 ft.) having an inclined base of asbestos board which facilitated rapid drainage of leachings into a gutter of the same material leading to a large collecting jar. A layer of

broken brick ($\frac{1}{2}$ in. deep) was laid at the bottom of each tray, and this was covered with a mixture of garden soil and sand (tray *A* contained 2 parts soil to 1 part sand to a depth of 4–5 in. and tray *B* 4 parts soil to 1 of sand). The seeds were sown in April 1943, in rows 5 in. apart, and the seedlings thinned out 6 weeks later, leaving about 120–130 plants in each tray. Towards the end of June, when the plants were 4 in. high and growing vigorously, they were well watered and a sample of leachings tested for activity by the method already described (Calam *et al.*, 1949). The leachings proved to be highly active, hatching 683 larvae from 60 cysts during 3 days. It was therefore decided to begin regular leaching of the plants at once.

The plants were leached early in the morning on alternate days, and the leachings treated at once with activated charcoal (British Drug Houses Ltd., 'for decolorizing purposes', 1 g./l.), shaken and left to settle. On the following day the bulk of the liquid was decanted from the charcoal, which was then sucked fairly dry on a Buchner funnel. The moist charcoal was allowed to accumulate until the end of each week and was then eluted with aqueous acetone. Watering was controlled so as to give about 3–4 l. of leachings from each tray every alternate day. In actual practice, however, this volume was rather variable as the plants were unprotected from the weather, and during rainy periods the trays were liable to be almost completely depleted of their content of hatching factor. The leachings were a very pale yellow and had pH 7.

RESULTS

Elution of charcoal

The accumulated moist charcoal on which the active factor was adsorbed was eluted each week with aqueous acetone (70 parts acetone to 30 parts water). About 40 g. of charcoal were stirred four times with 100 ml. aqueous acetone and the yellow filtrate concentrated below 30° under reduced pressure, and finally evaporated to dryness in a desiccator. The

crude solid eluate was a brown resinous powder and had a high activity when tested against an arbitrarily chosen 'standard solid' eluate from tomato plants (cf. Calam *et al.* 1949). As will be seen from Table 1 the Relative Activity (R.A.) of the crude eluate from tray A was very high during the early period of growth (R.A. 20-30) and gradually decreased after the plants had reached maturity. The weight yield of crude eluate per litre of leachings also showed a gradual decrease throughout the experiment, except in the last recorded week which showed a large increase in weight yield. As this increase occurred in the case of both trays at the same date, it was probably due to the exceptionally heavy rainfall during the previous week. The wet weather during September brought the experiment to a premature close, although it is doubtful whether much more could have been expected from the plants even if the weather had been more favourable.

The yield of crude solid from tray A was better in weight and activity than that from tray B (Tables 1 and 2). This may have been due to a variety of

Concentration of hatching factor

The purification of the crude solid eluate was carried out by applying the procedure which had been developed for the concentration of root excretion of tomato plants by Marrian *et al.* (1949).

The crude solid (2.3 g.) was dissolved in water, acidified with dilute H_2SO_4 and extracted continuously with peroxide-free ether for 12 hr. The moist ether extract (150 ml.) was put directly on to a column prepared from 20 g. silica gel moistened with 8 ml. 30% phosphate buffer, pH 6.0. After washing with wet ether (150 ml.) the column was drained and eluted by stirring with aqueous acetone (1:1). Evaporation of the ether runnings and washings gave 90 mg. of sweet smelling oil, probably terpene material, while the acetone eluate yielded 75 mg. of clear yellow resin, R.A. 150.

Brucine salt fractionation. The active concentrate from the chromatogram was dissolved in water (10 ml.) and shaken with brucine (250 mg.). The undissolved brucine (100 mg.) was filtered off, and the filtrate extracted by shaking several times with $CHCl_3$. Evaporation of the $CHCl_3$ extract gave 220 mg. brucine salts which were dissolved in ethanol (2.5 ml.) and a little insoluble matter spun off. Addition of ether

Table 1. *Details of hatching factor production from Solanum nigrum (tray A)*

Week ending	Volume of leachings (l.)	Wt. moist charcoal (g.)	Wt. crude eluate (mg.)	R.A.	Wt. crude eluate (mg./l.)
8 July 1943	11.5	42	205	24	18
17 July 1943	12	28	130	28	11
23 July 1943	13.5	38	160	32	12
31 July 1943	15.5	41	133	10	9
6 Aug. 1943	18	42	158	20	9
14 Aug. 1943	18	50	161	7	9
25 Aug. 1943	27.5	75	173	7	6
(Heavy rain all week ending 1 Sept 1943)					
13 Sept. 1943	19	50	238	11	13
Total			1358		

Table 2. *Details of hatching factor production from Solanum nigrum (tray B)*

Week ending	Volume of leachings (l.)	Wt. moist charcoal (g.)	Wt. crude eluate (mg.)	R.A.	Wt. crude eluate (mg./l.)
24 July 1943	9	26	123	—	14
30 July 1943	17	57	183	18	11
6 Aug. 1943	11	26	103	10	9
14 Aug. 1943	14.5	40	137	3	9
25 Aug. 1943	27.5	81	156	3	6
(Heavy rain all week ending 1 Sept. 1943)					
13 Sept. 1943	17	55	241	5	14
Total			943		

reasons. Tray A was planted some 3 weeks earlier than tray B and contained rather more plants. The plants in tray A therefore had a more favourable growing period as the later part of the summer was rather wet and cold. Another difference between the two trays was that B contained less sand than A, and consequently drained more slowly. The total yield of crude eluate obtained from both trays (251 plants) during the experiment was 2.3 g.

caused no precipitate, so a large excess of light petroleum was added (20 ml.) and the precipitated brucine salt (63 mg.) was collected and analysed, R.A. 35. (Found in material dried *in vacuo* over P_2O_5 at 100° : C, 65.1; H, 6.5; N, 5.8%. Average value for corresponding product from tomatoes: C, 63.4; H, 6.6; N, 6.1%.)

Preparation of the inactivated free acid. The purified brucine salt (60 mg.) was dissolved in water, acidified with dilute H_2SO_4 and extracted continuously with ether for 20 hr. Evaporation of the ether extract left 16 mg. of free acid which

was inactive. (Found in material dried *in vacuo* over P_2O_5 at 50°: C, 60.0; H, 6.1; N, 2.0%. Average value for corresponding product from tomatoes: C, 56.4; H, 6.1; N, 1.8%.)

CONCLUSIONS

The growing of *Solanum nigrum* out of doors with the minimum amount of attention yielded a root excretion which was highly active in hatching larvae of the potato eelworm from their cysts. Unfortunately, the rapid growth and short growing period of the plants did not permit more than about 9 weeks' leachings to be collected. The active principle from *S. nigrum* root excretion could be concentrated by the same procedure as was used for tomato root excretion, but the limited number of analyses on the brucine salt and free acid did not justify the deduction that the active principle from *S. nigrum* was identical with that obtained from tomatoes. Nevertheless, making allowance for the

non-crystalline character of the products, the great similarity in general properties would make it appear probable that the *S. nigrum* factor is either identical with that obtained from tomatoes or some very closely related substance.

SUMMARY

1. *Solanum nigrum* grown out of doors yielded a root excretion which was highly active in hatching potato eelworm cysts.

2. Concentration of the active material in this excretion gave a product which was closely similar to the corresponding product from tomato root excretion in its properties. The active principle in the two cases may well be identical.

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The Potato Eelworm Hatching Factor

5. ATTEMPTS TO PREPARE ARTIFICIAL HATCHING AGENTS.

PART I. SOME FURAN DERIVATIVES

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From the time when it became clear that highly active concentrates of the potato eelworm hatching factor prepared from tomato root excretion possesses both acidic and lactonic properties (Calam, Todd & Waring, 1949), synthetic experiments have been carried out alongside work on the natural factor in the hope that simpler substances might be prepared which would show hatching activity and which might be of practical interest in controlling eelworm infestation in potato crops. The examination of a number of tetrone acid derivatives has been described in a previous paper (Calam, Todd & Waring, 1949); the present communication deals with experiments on the synthesis of a number of lactones containing furan nuclei, such compounds being of interest, since it seemed probable that the natural hatching factor contained heterocyclic oxygen.

In commencing studies in the furan series an attempt was made to oxidize furitaconic acid (furfurylidenesuccinic acid) to β -hydroxy- β -carboxy- γ -2-furylbutanolide by means of potassium permanganate or monoperphthalic acid without result; this was unexpected in view of the ease with which itaconic acid yields hydroxyparaconic acid on similar treatment. Condensation of furfural with laevulinic acid in presence of sodium acetate gives a 10% yield of β -furfurylidene-laevulinic acid (Kehrer & Kleberg, 1893). Replacement of sodium acetate by piperidine failed to give any condensation and sodium hydroxide gave sticky products; with potassium acetate, however, yields of about 30% were obtained. No condensation occurred between furfural and ethyl laevulinate in presence of piperidine, but with sodium hydroxide β -furfurylidene-