

coenzymes, it will be theoretically possible to demonstrate this activity factor at any of the isolated steps. Technically, the sequence cytochrome *c* → cytochrome oxidase → oxygen is quite simple, because in homogenized roots the normal anion respiration is rapidly brought to a standstill, whereas the oxidase remains active as shown from its rapid oxidation of added reduced cytochrome *c*. The following experiment may serve as an illustration.

100 mgm. dry weight of wheat roots from desalted seedlings three weeks old was ground in a porcelain mortar after adding 10 c.c. distilled water. 1.5 c.c. of this preparation was added to a solution of 0.02 per cent cytochrome *c* ('Sigma'), reduced with some dithionite and afterwards aerated. The oxidation was followed spectrophotometrically by observing the decrease of the difference in extinction between the wavelengths 550 and 560 μ . After 5 min., 41 per cent of the cytochrome was oxidized. If 0.1 *M* potassium chloride was added to the preparation, 73 per cent of the cytochrome was oxidized in 5 min. Controls with 0.001 *M* hydrogen cyanide showed no oxidation. The figures correspond to an oxidation of 1.07 μ gm. iron per hr. per 10 mgm. in the desalted material and an oxidation of 1.70 μ gm. iron after addition of potassium chloride; thus there was an increase of 64 per cent due to the salt effect. Similar results were obtained with potassium nitrate. Desalting can also be brought about by treatment with anion-absorbing resin.

Simultaneous spectroscopic measurements on living roots showed a quantity of 3×10^{-6} μ mol. cytochrome oxidase per 10 mgm. dry weight. In the presence of salts, this quantity oxidized 1.7 μ mol. iron in 1 hr., corresponding to a turnover number of c. 1,000 per min. In the absence of salts this number decreases to c. 600; but this is, of course, not a minimum value because salts are always to some extent still present in 'desalted roots' (cf. ref. 2). In other experiments the activity of the preparations nearly equalled the normal activity of living roots, showing¹ a turnover number of c. 1,700 per min. at 19° C. The roots used for these experiments showed an active salt absorption from 0.0005 *M* potassium chloride of 0.82 μ mol. chlorine per hr. per 10 mgm., corresponding to $Q_{an}/Q_{O_2} = c. 0.9$ if calculated from the directly determined oxidase activity. This value is quite in accord with determinations of the anion respiration of living roots (see ref. 2).

H. LUNDEGÅRDH

Institute of Plant Physiology,
Uppsala 7. Dec. 29.

¹ Lundegårdh, H., *Nature*, **169**, 1088 (1952); *Ark. f. Kemi* (Swed. Acad. Sci.), **5**, No. 7 (1952), No. 12 (1953); and earlier papers.

² Lundegårdh, H., *Ann. Agric. Coll. Sweden*, **16**, 372 (1949); and earlier papers.

Acetic Acid in Fresh Grass

EXPERIMENTS carried out in these laboratories¹ on the formation of volatile fatty acids in grass/water slurries showed that, whereas butyric and propionic acids were formed after the primary production of lactic acid had been stabilized, acetic acid was produced even before the lactic fermentation began. It is, of course, well known that acetic acid is formed during the silage fermentation, but it seemed surprising that it should be formed so speedily.

An examination of a slurry which had been standing for two hours showed that appreciable amounts of acetic acid were even then present, although tests

for lactic acid by the *p*-hydroxy diphenyl test¹⁻³ gave negative results.

It was decided to experiment with fresh grass juice; for this purpose, samples of grass obtained from a plot adjoining the laboratory were immediately minced and the product squeezed through a linen cloth which had been washed in hot water and then dried. The grass juice was subjected to steam distillation according to the procedure of Scarisbrick⁴ to isolate the total volatile fatty acids, and the distillate was submitted to gas chromatography according to the technique of James and Martin⁵. A definite yield (about 8 mgm. per cent of the grass dry matter) was obtained on each occasion, but no other lower fatty acid in the C₁-C₁₂ range was isolated.

So far as we are aware, such a finding has not previously been reported in the literature, and all that may be concluded at this stage is that acetic acid is present, either free or in a state of loose combination, in the grass. It is possible that it forms an intermediate in the tricarboxylic acid cycle.

A. J. G. BARNETT

R. E. B. DUNCAN

Division of Agricultural Biochemistry,
Department of Biological Chemistry,
University of Aberdeen.
Sept. 18.

¹ Barnett, A. J. G., and Duncan, R. E. B., *Biochem. J.*, **52**, xvii (1952).

² Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, **138**, 535 (1941).

³ Barnett, A. J. G., *Biochem. J.*, **40**, 527 (1951).

⁴ Scarisbrick, R., *Biochem. J.*, **xxxiv** (1952).

⁵ James, A. J., and Martin, A. J. P., *Biochem. J.*, **50**, 679 (1952).

Hatching Responses in Root Eelworms (*Heterodera* spp.)

RESULTS of recent investigations on larval emergence from cysts of root eelworms (*Heterodera* spp.) when exposed to plant root diffusates (using the methods evolved by Fenwick for potato root eelworm^{1,2,4}) are summarized below.

Beet, potato and carrot root eelworms behaved normally in that they responded only in diffusates from hosts and from invaded plants. Cabbage root eelworm responded well in diffusates from hosts belonging to the genus *Brassica*, but did not respond to other cruciferous hosts or to non-hosts. *Galeopsis* and clover eelworms gave little or no response in diffusates from typical hosts, but responded readily to pea root diffusate. Hop eelworm responded to diffusates from three hosts, hop, hemp and small nettle (*Urtica urens*), and from the related plant *Parietaria diffusa*. Negligible responses in host and non-host diffusates were obtained with pea and oat root eelworms.

From the above findings, it seemed that a measure of success might be obtained in quantitative estimation of mixed populations of *Heterodera* species by exposing the cysts to a succession of plant root diffusates. In preliminary experiments of this nature reliable estimates (in terms of 'hatchable' larvae) were obtained for a mixed soil population of beet, cabbage and *Galeopsis* eelworm cysts (see graph). A limitation of the method is that it is not applicable to those species which do not respond to root diffusates, for example, pea and oat root eelworms.

Applying Fenwick's technique for determining the shape of the hatching curve³ to some of the above-mentioned species, it has been found that in all cases investigated (those of beet, cabbage, *Galeopsis* and